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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 5 : C07K 7/54, 7/56

A1

(11) International Publication Number: WO 91/01331

(43) International Publication Date: 7 February 1991 (07.02.91)

(21) International Application Number: PCT/US90/03788

(22) International Filing Date: 5 July 1990 (05.07.90)

(30) Priority data:
380,957 17 July 1989 (17.07.89) US

(60) Parent Application or Grant
(63) Related by Continuation
US 380,957 (CIP)
Filed on 17 July 1989 (17.07.89)

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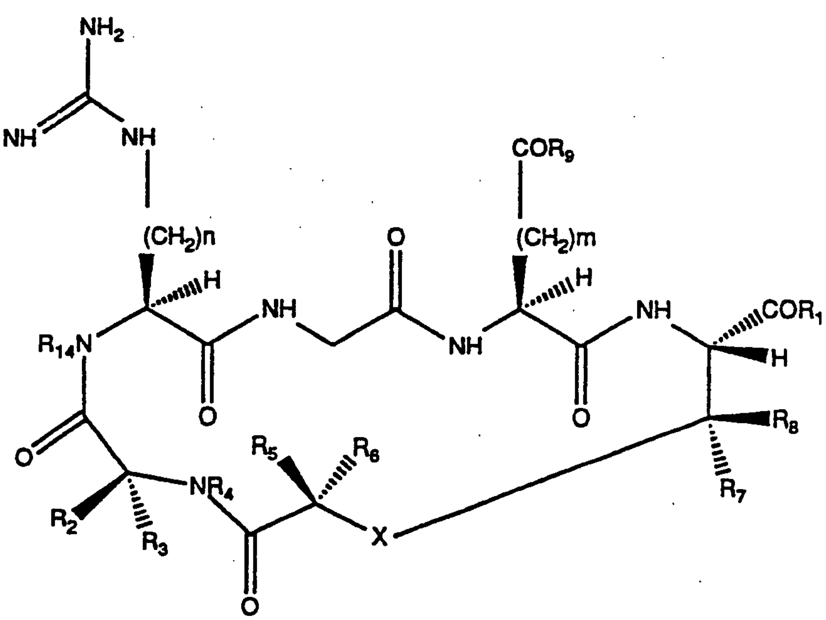
(81) Designated States: AT (European patent), AU, BE (European patent), CA, CH (European patent), DE (European patent)*, DK (European patent), ES (European patent), FR (European patent), GB (European patent), IT (European patent), JP, LU (European patent), NL (European patent), SE (European patent), US.

Published
With international search report.

(54) Title: SMALL CYCLIC PLATELET AGGREGATION INHIBITORS



(I)

(57) Abstract

A platelet aggregation inhibitor useful as an antithrombotic is provided which consists of a synthetic cyclic pentapeptide containing the tripeptide sequence Arg-Gly-Asp and a thioether linkage in the cycle. A preferred platelet aggregation inhibitor is represented by formula (I), where R_1 and R_9 are OH; R_2, R_4, R_5, R_6, R_7, R_8 and R_{14} are hydrogen; R_3 and R_4 are joined to form a pyrrolidine ring; X is sulfur; m is 1 and n is 3.

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SMALL CYCLIC PLATELET AGGREGATION INHIBITORS

Field of the Invention

The present invention relates to inhibitors of platelet aggregation. Specifically, the invention is directed to antagonists of the final common pathway of platelet aggregation and that as potent antithrombotics. The invention further relates to therapeutic applications of these inhibitors in diseases for which blocking of platelet aggregation and intracellular adhesion is indicated.

Background of the Invention

Platelets are particles found in whole blood which participate in the process of thrombus formation and blood coagulation. A membrane bound glycoprotein, commonly known as GP IIbIIIa, is present on the exterior surface of platelets. Glycoprotein IIbIIIa is a non-covalent, calcium ion dependent heterodimer complex composed of alpha and beta subunits (Jennings, et al., *J. Biol. Chem.* (1982) 257, 10458). These glycoproteins contribute to normal platelet function through interactions with proteins containing the amino acid sequence Arg-Gly-Asp, such as fibrinogen. The interaction of GP IIbIIIa with fibrinogen is stimulated by certain factors released or exposed when a blood vessel is injured. Multiple factors, including a variety of physiologic stimuli and soluble mediators, initiate platelet activation via several pathways. These pathways have a common final step which is the activation of the GP IIbIIIa receptor on the platelet surface and its subsequent binding to fibrinogen followed by aggregation and thrombus formation. By virtue of these interactions GP IIbIIIa is a component of the platelet aggregation system (Pytela et al., *Science* (1986) 231, 1559). Thus, inhibition of the interaction of GP IIbIIIa with Arg-Gly-Asp containing ligands such as fibrinogen is a useful means of modulating thrombus formation. An inhibitor which prevents this binding interaction would antagonize platelet activation by any stimulus and therefore would have important antithrombotic properties.

Many common human disorders are characteristically associated with a hyperthrombotic state leading to intravascular thrombi and emboli. These are a major cause of medical morbidity, leading to infarction, stroke and phlebitis and of mortality from stroke and pulmonary and cardiac emboli. Patients with atherosclerosis are predisposed to arterial thromboembolic phenomena for a variety of reasons. Atherosclerotic plaques form niduses for platelet plugs and thrombii that lead to vascular narrowing and occlusion, resulting in myocardial and cerebral ischemic disease. This may happen spontaneously or following procedures such as angioplasty or endarteroectomy. Thrombii that break off and are released into the circulation cause infarction of different organs, especially the brain, extremities, heart and kidneys.

In addition to being involved in arterial thrombosis, platelets may also play a role in venous thrombosis. A large percentage of such patients have no antecedent risk factors and develop venous thrombophlebitis and subsequent pulmonary emboli without a known cause. Other patients who form venous thrombi have underlying diseases known to predispose to these syndromes. Some of these patients may have genetic or acquired deficiencies of factors that normally prevent hypercoagulability, such as antithrombin-3. Others have mechanical obstructions to venous flow, such as tumor masses, that lead to low flow states and thrombosis. Patients with

malignancy have a high incidence of thrombotic phenomena for unclear reasons. Antithrombotic therapy in this situation with currently available agents is dangerous and often ineffective.

Patients whose blood flows over artificial surfaces, such as prosthetic synthetic cardiac valves or through extracorporeal perfusion devices, are also at risk for the development of platelet plugs, thrombii and emboli. It is standard practice that patients with artificial cardiac valves be chronically anti-coagulated. However, in all instances, platelet activation and emboli formation may still occur despite adequate anticoagulation treatment.

Thus, a large category of patients, including those with atherosclerosis, coronary artery disease, artificial heart valves, cancer, and a history of stroke, phlebitis, or pulmonary emboli, are candidates for limited or chronic antithrombotic therapy. The number of available therapeutic agents is limited and these, for the most part, act by inhibiting or reducing levels of circulating clotting factors. These agents are frequently not effective against the patient's underlying hematologic problem, which often concerns an increased propensity for platelet aggregation and adhesion. They also cause the patient to be susceptible to abnormal bleeding. Available antiplatelet agents, such as aspirin, inhibit only part of the platelet activation process and are therefore often inadequate for therapy.

An agent which effectively inhibits the final common pathway of platelet activation, namely fibrinogen binding to the GP IIbIIIa receptor, should accordingly be useful in a large group of disorders characterized by a hyperthrombotic state as described above. The present invention contemplates such an agent which is a new composition, namely a cyclic polypeptide consisting in part of natural amino acids and in part of unnatural amino acids. This new composition interferes with the interaction of Arg-Gly-Asp containing peptides, particularly fibrinogen, with the GP IIbIIIa complex thereby preventing platelet aggregation. Platelet aggregation has been identified as an early step in the formation of platelet plugs, emboli and thrombii in the circulatory system which in turn have been shown to play an active role in cardiovascular complications and disease. Inhibition of fibrinogen binding to the GP IIbIIIa complex has been shown to be an effective antithrombotic treatment in animals (H. K. Gold, et al., *Circulation* (1988) 77, 670-677; T. Yasuda, et al., *J. Clin. Invest.* (1988) 81, 1284-1291; B. S. Coller, et al., *Blood* (1986) 68, 783-786.)

Other proteins such as fibronectin contain the Arg-Gly-Asp sequence of amino acids. Large polypeptide fragments of fibronectin have been shown to have activity for cell attachment to various surfaces which has been disclosed in U.S. Patents 4,517,686; 4,589,881; and 4,661,111. These large polypeptides contain the amino acid sequence Arg-Gly-Asp-Ser in the interior portion of the polypeptide chain. Short peptides derived from the large polypeptides were also found to promote cell attachment to various substrates when bound on the substrate. Alternatively, the same short peptides were found to inhibit cell attachment to the same substrates when dissolved or suspended in the medium surrounding the substrate. This activity has been disclosed in U.S. Patents 4,578,079, 4,614,517 and 4,792,525. The short peptides were defined as

Q-Arg-Gly-Asp-AA1-B

where Q is hydrogen or an amino acid; AA1 is serine, threonine, or cysteine; and B is hydroxy or an amino acid. No discussion of cyclizing these short peptides is presented.

A number of synthetic peptides, including cyclic disulfides, have been disclosed as inhibitors of fibrinogen binding to platelets all of which contain the Arg-Gly-Asp sequence. See U.S. Patent 4,683,291;

WO89/05150; EPO 0 319 506 A2; EPO 0 341 915 A2; Plow et al., *Proc. Natl. Acad. Sci. USA* (1985) 82, 8057-8061; Ruggeri et al., *Proc. Natl. Acad. Sci. USA* (1986) 83, 5708-5712; Haverstick et al., *Blood* (1985) 66, 946-952; Plow et al., *Blood* (1987) 70, 110-115; F. El F. Ali, et al., *Proc. Eleventh Amer: Peptide Symp.* (1990) 94-96; M. Pierschbacher and E. Ruoslahti, *J. Biol. Chem.* (1987) 262, 17294-17298; and references cited in the above publications.

Several synthetic cyclic peptides containing the thioether linkage have been synthesized. Gero et al., *Biochem. Biophys. Res. Comm.* (1984) 120, 840-845 describe a pseudohexapeptide analog of somatostatin where the group [CH_2-S] is substituted for a peptide bond. Similarly, Edwards et al., *Biochem. Biophys. Res. Comm.* (1986) 136, 730-736 compare the biological activity of linear and cyclic enkephalin pseudopeptide analogs containing the thiomethylene ether linkage. Other enkephalin related pseudopeptides and macrocycles containing the [CH_2-S] substitution for peptides have been described, Spatola et al., *Biopolymers* (1986) 25, 229-244 and Spatola et al., *Tetrahedron* (1988) 44, 821-833.

None of these references disclose a small cyclic peptide containing a stable ring structure having high platelet aggregation inhibition activity.

Accordingly, it is an object of this invention to produce a small cyclic peptide having high platelet aggregation inhibition activity. It is a further object to produce such small cyclic peptides that are stable to ring opening. It is still a further object of this invention to provide a platelet aggregation inhibitor having a long *in vivo* lifetime.

These and other objects of this invention will be apparent from consideration of the invention as a whole.

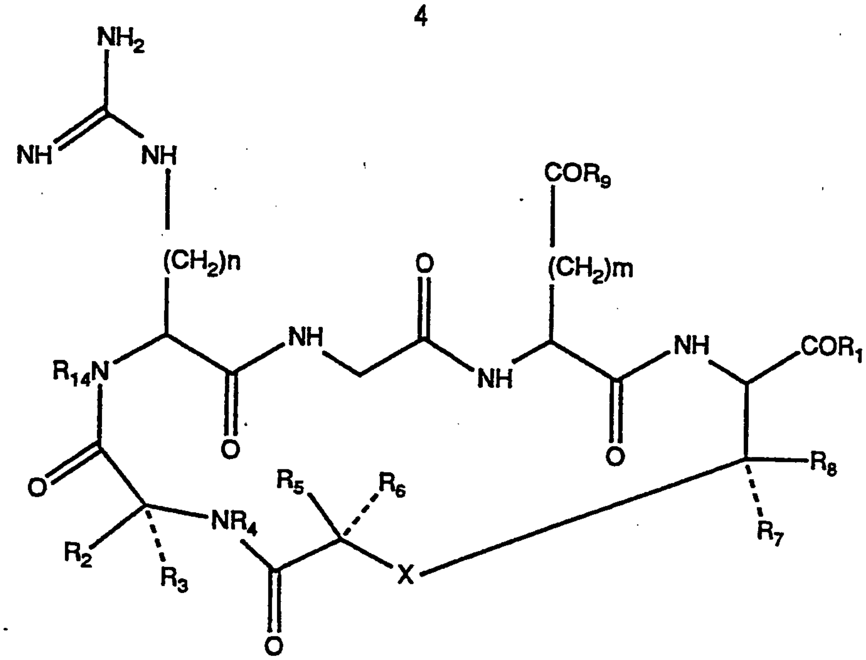
Summary of the Invention

The objects of this invention are accomplished by providing a small cyclic peptide containing the tripeptide sequence Arg-Gly-Asp and containing a thioether, sulfoxide or sidechain amide bond within the cycle. Preferably, the cyclic peptide has about 5 amino acids forming the ring of the cycle. More preferably, the ring of the cyclic peptide contains from about 17 to about 18 atoms, most preferably 18 atoms.

Also preferably, the ring will contain a D amino acid most preferably linked to the Arg of the tripeptide sequence.

The preferred and most preferred compounds of the instant invention are represented by Formulae I and Ia below.

The invention in its broad aspects relates to peptide derivatives which are useful as inhibitors platelet function mediated by the GP IIbIIIa receptor and for the prevention of thrombus formation. Preferred compounds of this invention are represented by Formula I:



Formula I

wherein

R_1 and R_9 are the same or different and are selected from

hydroxy,

C_1-C_8 alkoxy,

C_3-C_{12} alkenoxy,

C_6-C_{12} aryloxy,

di-C_1-C_8 alkylamino-C_1-C_8-alkoxy,

acylamino-C_1-C_8-alkoxy selected from the group acetylaminoethoxy, nicotinoylaminoethoxy, and succinamidoethoxy,

pivaloyloxyethoxy,

C_6-C_{12} aryl-C_1-C_8-alkoxy where the aryl group is unsubstituted or substituted with one or more of the groups nitro, halo (F, Cl, Br, I), C_1-C_4-alkoxy, and amino,

hydroxy-C_2-C_8-alkoxy,

dihydroxy-C_3-C_8-alkoxy, and

$NR_{10}R_{11}$ wherein R_{10} and R_{11} are the same or different and are hydrogen, C_1-C_8-alkyl, C_3-C_8-alkenyl, C_6-C_{12}-aryl where the aryl group is unsubstituted or substituted with one or more of the groups

nitro, halo (F, Cl, Br, I), C_1-C_4-alkoxy, and amino, C_6-C_{12}-aryl-C_1-C_8-alkyl where the aryl group is unsubstituted or substituted by one or more of the groups nitro, halo (F, Cl, Br, I), C_1-C_4-alkoxy, and amino;

R_2, R_3, R_5, R_6, R_7, R_8 are the same or different and are selected from

hydrogen,

C_6-C_{12} aryl where the aryl group is unsubstituted or substituted by one or more of the groups nitro, hydroxy, halo (F, Cl, Br, I), C_1-C_8 alkyl, halo-C_1-C_8 alkyl, C_1-C_8-alkoxy, amino, phenyloxy, phenyl, acetamido, benzamido, di-C_1-C_8 alkylamino, C_1-C_8 alkylamino, C_6-C_{12} aroyl, C_1-C_8 alkanoyl, and hydroxy-C_1-C_8 alkyl,

C_1-C_{12} alkyl either substituted or unsubstituted, branched or straight chain where the substituents are selected from halo (F, Cl, Br, I),

C_1-C_8 alkoxy,

C_6-C_{12} aryloxy where the aryl group is unsubstituted or substituted by one or more of the groups nitro, hydroxy, halo (F, Cl, Br, I), C_1-C_8 alkyl, C_1-C_8-alkoxy, amino, phenyloxy, acetamido, benzamido, di-C_1-C_8 alkylamino, C_1-C_8 alkylamino, C_6-C_{12} aroyl, and C_1-C_8 alkanoyl,

isothioureido,

C_3-C_7 cycloalkyl,

ureido,

amino,

C_1-C_8 alkylamino,

di-C_1-C_8 alkylamino,

hydroxy,

amino-C_2-C_8 alkylthio,

amino-C_2-C_8 alkoxy,

acetamido,

benzamido wherein the phenyl ring is unsubstituted or substituted by one or more of the groups nitro, hydroxy, halo (F, Cl, Br, I), C_1-C_8 alkyl, C_1-C_8-alkoxy, amino, phenyloxy, acetamido, benzamido, di-C_1-C_8 alkylamino, C_1-C_8 alkylamino, C_6-C_{12} aroyl, and C_1-C_8 alkanoyl,

C_6-C_{12} arylamino wherein the aryl group is unsubstituted or substituted by one or more of the groups nitro, hydroxy, halo, C_1-C_8 alkyl, C_1-C_8-alkoxy, amino, phenyloxy, acetamido, benzamido, di-C_1-C_8 alkylamino, C_1-C_8 alkylamino, C_6-C_{12} aroyl, and C_1-C_8 alkanoyl,

guanidino,

phthalimido,

mercapto,

C_1-C_8 alkylthio,

C_6-C_{12} arylthio,

carboxy,

carboxamide,

carbo-C_1-C_8 alkoxy,

C_6-C_{12} aryl wherein the aryl group is unsubstituted or substituted by one or more of the groups nitro, hydroxy, halo, C_1-C_8 alkyl, C_1-C_8-alkoxy, amino, phenyloxy, acetamido,

benzamido, di-C_1-C_8 alkylamino, C_1-C_8 alkylamino, hydroxy-C_1-C_8 alkyl, C_6-C_{12} aroyl, and C_1-C_8 alkanoyl, and

aromatic heterocycle wherein the heterocyclic groups have 5-10 ring atoms and contain up to two O, N, or S heteroatoms;

R_2 and R_3, R_5 and R_6, or R_7 and R_8 may optionally and independently be joined together to form a carbocyclic or heterocyclic ring of from four to seven atoms where the heteroatoms are selected from O, S or NR_{12} where R_{12} is selected from

hydrogen, C_1-C_8-alkyl, C_3-C_8-alkenyl, C_6-C_{12}-aryl, C_6-C_{12}-aryl-C_1-C_8-alkyl, C_1-C_8 alkanoyl, and C_6-C_{12} aroyl;

R_4 is selected from

hydrogen,

C_1-C_8 alkyl,

C_3-C_{10} cycloalkyl,

C_6-C_{12} aryl, and

C_6-C_{12} aryl-C_1-C_8-alkyl;

R_2 or R_3 may be optionally joined with R_4 to form a piperidine, pyrrolidine or thiazolidine ring;

R_{14} is selected from

hydrogen, C_1-C_8-alkyl, C_3-C_8-alkenyl, C_6-C_{12}-aryl, and C_6-C_{12} aryl-C_1-C_8-alkyl;

X is selected from

an O or S atom,

an S atom bearing one or two O atoms,

NR_{13} wherein R_{13} is hydrogen, C_1-C_8-alkyl, C_3-C_8-alkenyl, C_6-C_{12}-aryl, C_6-C_{12}-aryl-C_1-C_8-alkyl, C_1-C_8 alkanoyl, and C_6-C_{12} aroyl, and

C_6-C_{12} aryl,

C_1-C_8 alkanoyl,

$(CH_2)_k$ where k is an integer from 0 to 5;

n is an integer from 1 to 6;

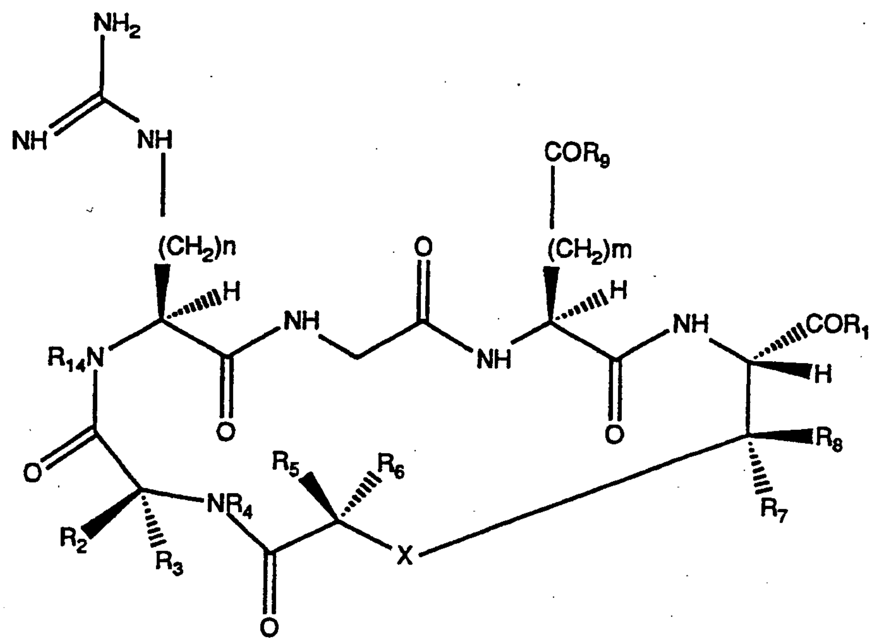
m is an integer from 0 to 4; and

pharmaceutically acceptable salts thereof.

As used herein and unless specified otherwise: alkyl, alkenyl and alkynyl denote straight and branched hydrocarbon chains having single, double and triple bonds, respectively; C_6-C_{12} aryl groups denote unsubstituted aromatic ring or fused rings such as, for example, phenyl or naphthyl; hetero denotes the heteroatoms O, N, or S; aromatic heterocyclic groups have 5-10 ring atoms and contain up to four heteroatoms; halogen or halo denote F, Cl Br, or I atoms; alkoxy denotes an alkyl group attached to O.

Examples of C_1-C_8 alkyl or C_2-C_8 alkenyl groups include methyl, ethyl, propyl, isopropyl, butyl, t-butyl, pentyl, isopentyl, hexyl, vinyl, allyl, butenyl and the like; examples of C_3-C_{10}-cycloalkyl groups include cyclopropyl, cyclopentyl, cyclohexyl, and the like; aromatic heterocyclic groups include but are not limited to pyridyl, thienyl, furyl, indolyl, benzthienyl, imidazolyl, thiazolyl, quinolinyl and isoquinolinyl.

Most preferred compounds of the instant invention are represented by Formula Ia:



Formula Ia

wherein

R_1 and R_9 are the same or different and are hydroxy, NH_2, C_1-C_4 alkoxy or benzyloxy;

R_2 is hydrogen

R_3 is selected from

- hydrogen,
- C_1-C_6 alkyl branched or unbranched, unsubstituted or substituted with substituents selected from amino, hydroxy, mercapto, methylthio, carboxy, carboxamide, guanidino, phenyl, 4-hydroxyphenyl, 4-methoxyphenyl, 3-indolyl, and 4-imidazolyl,
- phenyl either unsubstituted or substituted with one to three substituents that may be independently nitro, hydroxy, halo (F, Cl, Br, I), C_1-C_4 alkyl, C_1-C_4-alkoxy, amino, phenyloxy, phenyl, acetamido, benzamido, di-C_1-C_4 alkylamino, C_1-C_4 alkylamino, halo-C_1-C_4 alkyl, C_6-C_{12} aroyl, and C_1-C_4 alkanoyl,
- 1-naphthyl,
- 2-naphthyl,
- 2-thienyl,
- 2-pyridyl,
- 3-pyridyl, and
- 4-pyridyl;

R_5 and R_6 are independently selected from

- hydrogen,

C_1-C_6 alkyl, either branched or unbranched, unsubstituted or substituted with substituents selected from amino, hydroxy, mercapto, carboxy, carboxamide, guanidino, phenyl or 4-hydroxyphenyl, 4-methoxyphenyl, 3-indolyl, and 4-imidazolyl,

phenyl, either unsubstituted or substituted with one to three substituents that may be independently selected from nitro, hydroxy, halo (F, Cl, Br, I), C_1-C_4 alkyl, C_1-C_4-alkoxy, amino, phenyloxy, phenyl, acetamido, benzamido, di-C_1-C_4 alkylamino, C_1-C_4 alkylamino, halo-C_1-C_4 alkyl, C_6-C_{12} aroyl, and C_1-C_4 alkanoyl,

1-naphthyl,

2-naphthyl,

2-thienyl,

2-pyridyl,

3-pyridyl, and

4-pyridyl;

R_7 or R_8 are the same or different and are selected from

hydrogen,

C_1-C_4 alkyl,

phenyl either unsubstituted or substituted with from one to three substituents independently selected from hydroxy, halo (F, Cl, Br, I), C_1-C_4 alkyl, and C_1-C_4-alkoxy;

R_4 is hydrogen or may be joined with R_3 to form a heterocyclic ring selected from piperidine, pyrrolidine or thiazolidine;

R_{14} is hydrogen or methyl;

X is selected from

an O or S atom,

an S atom bearing one or two O atoms,

NR_{13} where R_{13} is selected from hydrogen, C_1-C_4 alkyl, benzyl, phenyl, C_1-C_4 alkanoyl, benzoyl and

$(CH_2)_k$, where k is 0 to 5;

n is 3 or 4;

m is 1; and

pharmaceutically acceptable salts thereof.

The present invention includes a method of making the compounds of Formulae I and Ia.

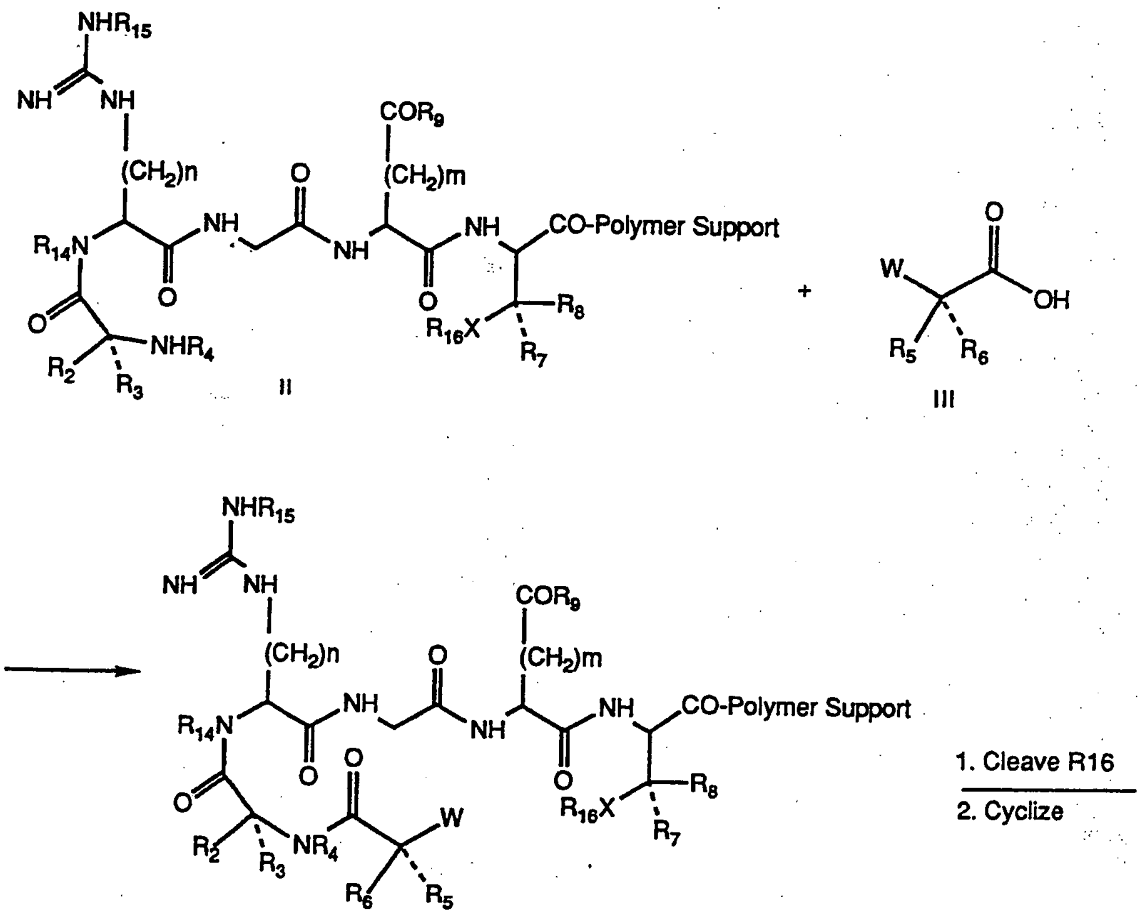
The present invention also includes a method for reducing platelet aggregation in a mammal. This method involves administering a therapeutically effective amount of the compounds of the present invention alone or in combination with a pharmacologically acceptable carrier. This general method may also be applied to treat a mammal having an increased propensity for thrombus formation.

Additionally, the present invention is directed to compositions of matter for reducing platelet aggregation in a mammal; treating a mammal having an increased propensity for thrombus formation; or inhibiting binding of a ligand to GP IIbIIIa in a mammal; wherein each of these compositions contains as an active ingredient one or more of the cyclic peptides defined in Formula I.

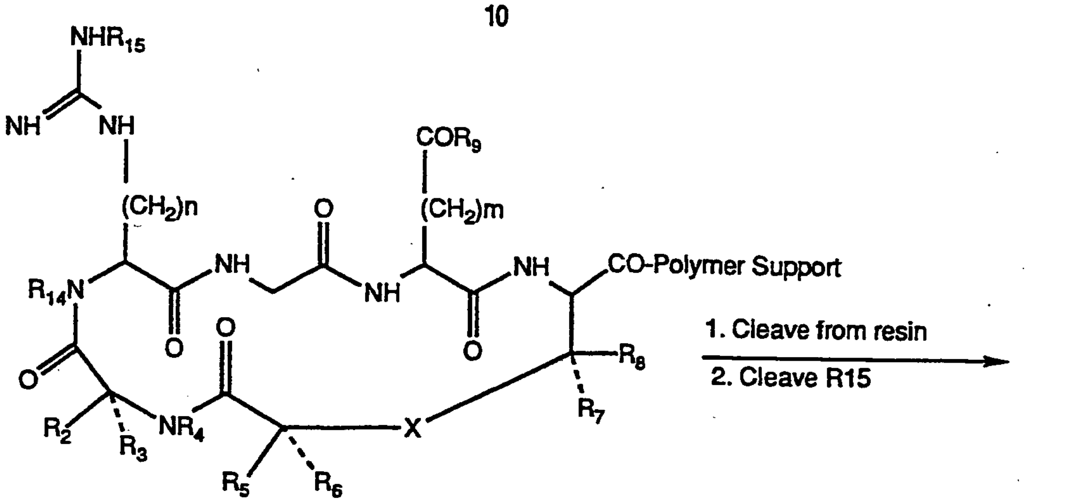
Detailed Description of the Invention

The products of Formula I and the preferred substituents can be made by using one of the methods depicted below or by other methods known in the art (see e.g., Spatola et al., *Tetrahedron* (1988) 44, 821-833, and references cited therein). The definitions of the substituent groups are the same as for Formula I except where noted.

Method A



IV



V

A peptide derivative bound to a polymer support, depicted by intermediate II, may be prepared by sequential coupling of individual amino acid derivatives by standard techniques. (Merrifield, R. B., *J. Am. Chem. Soc.* (1963) 85, 2149-2154; Stewart, J. M. and Young, J. D., *Solid Phase Peptide Synthesis* (1984), Pierce Chemical Co., Rockford, IL and additional references cited in the above publications). When the tetrapeptide derivative II is obtained, the terminal amino group is acylated with a suitable carboxylic acid derivative III. The acylation to yield IV may be accomplished using a number of standard methods which require activation of the carboxylic acid group of III. For example, activation may be obtained by the addition of an equimolar amount of dicyclohexylcarbodiimide or related carbodiimide reagent. If desired an additive such as 1-hydroxybenztriazole or N-hydroxysuccinimide may be incorporated. Alternatively, the carboxyl group may be activated by conversion to a halo derivative. For example, the chloride may be obtained by treatment of the acid with thionyl chloride or oxalyl chloride in a compatible solvent such as dichloromethane, toluene, or ethylene dichloride if desired. The substituent W is chosen such that it is readily displaceable by the group X. Suitable substituents W are, for example, halo atoms such as bromine or iodine or activated oxygen functions such as methanesulfonyloxy or p-toluensulfonyloxy and related sulfonic acid esters.

Cyclization to the resin bound intermediate V may be accomplished by selectively exposing the nucleophilic group X by removal of R_{16} and allowing X to react such that it displaces group W with formation of a new chemical bond. For example, if X is a sulfur or oxygen atom and R_{16} is a triphenylmethyl group, then R_{16} may be selectively cleaved from X using a very dilute solution of a strong acid such as trifluoroacetic acid in a solvent compatible with the polymer resin. Examples of resin compatible solvents are dimethylacetamide, dimethylformamide or dichloromethane and the like.

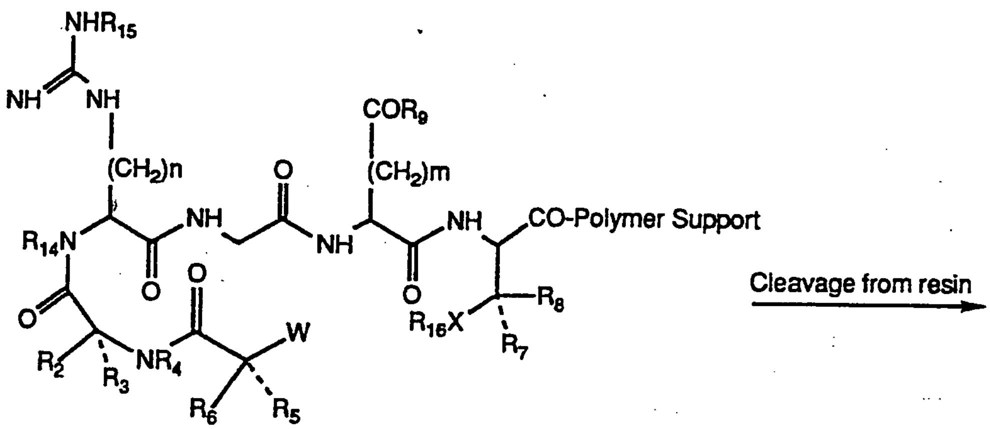
The end result of the cleavage process is replacement of the R_{16} group with a hydrogen atom. After cleavage of R_{16}, the resin bound peptide derivative V (R_{16} = H) is allowed to react in a suitable solvent such as dimethylacetamide until cyclization is complete. If desired, a base such as N-methylmorpholine may be incorporated into the reaction. Other protecting groups in the peptide molecule IV must be stable to the reaction conditions chosen to form V. For example, R_9 may be a group which affords an ester such as methoxy, ethoxy, benzyloxy, t-butyloxy and the like or an amide or substituted amide. R_{15} may be an arylsulfonyl group such as 2,2,5,7,8-pentamethylchroman-6-sulfonyl (PMC) or p-tolvenesulfonyl. Final cleavage of the cyclized peptide product from

the polymer resin may be accomplished in a variety of ways dependent upon the type of resin used and the chemical linkage between the cyclized peptide and the resin. If, for example, the resin is derived from a polymerized p-alkoxybenzyl alcohol derivative, then cleavage of the peptide-resin linkage may be carried out using a strong acid such as trifluoroacetic acid. If desired, additives such as phenol, anisole and ethanedithiol may be added to the reaction.

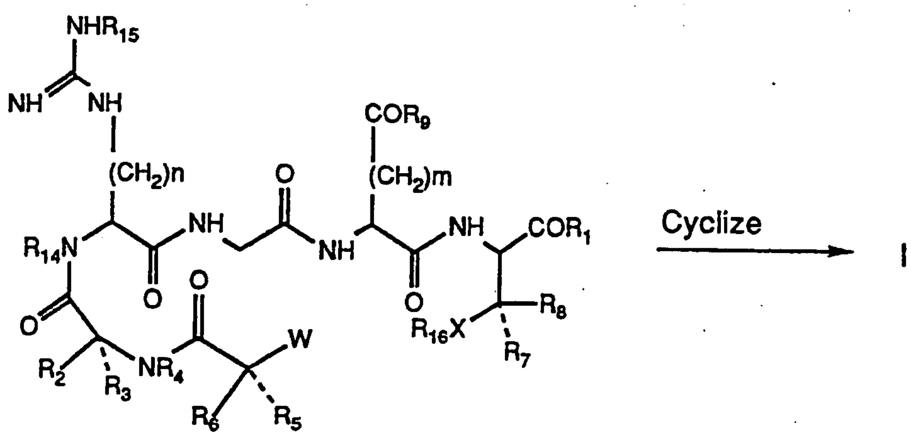
The groups R_9 and R_{15} may be chosen, if desired, to also be cleavable concurrently with cleavage of the cyclized peptide from the polymer resin. Examples of such chemical groups are R_9 = t-butyloxy, cleavage of which yields R_9 = OH and R_{15} = 2,2,5,7,8-pentamethylchroman-6-sulfonyl, cleavage of which affords R_{15} = H. The crude product thus obtained may be further purified using chromatographic or other methods of chemical purification to obtain I.

Further derivatization of I may be carried out if desired. For example, if X is S, treatment of I with a stoichiometric amount of an oxidizing agent such as 3-chloroperoxybenzoic acid or similar agent will produce the sulfoxide derivative where X is SO. Use of an excess amount of oxidant will afford the sulfone derivative where X is SO_2.

Method B



IV



VI

Alternatively, the linear peptide derivative IV, prepared as described above in Method A, may be cleaved from the resin prior to cyclization to yield VI. For example, if IV is synthesized on a polystyrene resin the cleavage can be accomplished using liquid hydrogen flouride. The groups R_9, R_{15} and R_{16} may, if desired, be cleaved concurrently under these conditions. If concurrent cleavage is desired, then examples of suitable substituents R_9 are t-butyloxy, benzyloxy or cyclohexyloxy, R_{15} is p-toluenesulfonyl or 2,2,5,7,8-pentamethylchroman-6-sulfonyl and R_{16} is triphenylmethyl or p-methylbenzyl if X is either O or S, or t-butoxycarbonyl if X is NR_{13}. Cleavage of these groups would result in R_9 being OH and R_{15} and R_{16} being hydrogen. The peptide derivative VI may then be cyclized in solution in the presence of a weak base such as ammonium hydroxide. The group W is as described in Method A. The resulting crude I may then be purified as described above in Method A.

The purified I may be further transformed as described in Method A. Additionally and if desired, when X is NR_{13} and R_{13} is hydrogen, I may be acylated with, for example, acetyl chloride, acetic anhydride or benzoyl chloride, methanesulfonyl chloride or p-toluenesulfonyl chloride and the like.

Method C

Intermediate VI may be prepared by the sequential coupling of amino acid derivatives in solution without the use of polymer resin or other solid supports. The methods useful for solution phase peptide synthesis are well documented in the chemical literature and are known to those skilled in the art (Houben-Weyl, Methoden der Organischen Chemie, 4th Edn., Vol. 15, Georg Thieme Verlag, Stuttgart 1974). The attached substituents R_1, R_9, R_{15} and R_{16} may be chosen such that they are transformable concurrently or sequentially as described in Methods A and B above. Cyclization of of VI wherein R_{16} is H under conditions described above in Method B will provide compounds of Formula I.

The starting materials required for the processes described herein are known in the literature or can be prepared using known methods and known starting materials.

Isomeric Products

In products of Formula I carbon atoms bonded to four nonidentical substituents are asymmetric. Accordingly, the compounds may exist as diastereoisomers, enantiomers or mixtures thereof. The syntheses described above may employ racemates, enantiomers or diastereomers as starting materials or intermediates. Diastereomeric products resulting from such syntheses may be separated by chromatographic or crystallization methods. Likewise, enantiomeric product mixtures may be separated using the same techniques or by other methods known in the art. Each of the asymmetric carbon atoms, when present in compounds of Formula I, may be in one of two configurations (R or S) and both are within the scope of the present invention. The carbon atoms bearing the $(CH_2)_n$ sidechain and the $(CH_2)_m$ sidechain are generally preferred to have the S configuration. The carbon atom bearing the substituents R_2 and R_3 is generally preferred to have a configuration corresponding to that of a D amino acid. The configuration may be assigned R or S depending on the chemical composition of R_2 and R_3.

The compounds described in this invention may be isolated as the free acid or base or converted to salts of various inorganic and organic acids and bases. Such salts are within the scope of this invention. Examples of such salts include ammonium, metal salts like sodium, potassium, calcium and magnesium; salts with organic bases like dicyclohexylamine, N-methyl-D-glucamine and the like; and salts with amino acids like arginine or lysine. Salts with inorganic and organic acids may be likewise prepared, for example, using hydrochloric, hydrobromic, sulfuric,

phosphoric, trifluoroacetic, methanesulfonic, malic, maleic, fumaric and the like. Non-toxic and physiologically compatible salts are particularly useful although other less desirable salts may have use in the processes of isolation and purification.

A number of methods are useful for the preparation of the salts described above and are known to those skilled in the art. For example, reaction of the free acid or free base form of a compound of Formula I with one or more molar equivalents of the desired acid or base in a solvent or solvent mixture in which the salt is insoluble; or in a solvent like water after which the solvent is removed by evaporation, distillation or freeze drying. Alternatively, the free acid or base form of the product may be passed over an ion exchange resin to form the desired salt or one salt form of the product may be converted to another using the same general process.

The compounds described in the present invention inhibit the binding of fibrinogen to its receptor on platelets, GP IIbIIIa, and thus prevent the aggregation of platelets and the formation of platelet plugs, emboli and thrombii in the circulatory system in mammals. Thromboembolic disorders have been shown to be directly related to the susceptibility of blood platelets to aggregate. Mammals exposed to medical procedures such as angioplasty and thrombolytic therapy are particularly susceptible to thrombus formation. The compounds of the present invention can be used to inhibit thrombus formation following angioplasty. They may also be used in combination with thrombolytic agents such as tissue plasminogen activator and its derivatives (US patents 4,752,603; 4,766,075; 4,777,043; EP 199,574; EP 0238,304; EP 228,862; EP 297,860; PCT WO89/04368; PCT WO89/00197), streptokinase and its derivatives, or urokinase and its derivatives to prevent arterial reocclusion following thrombolytic therapy. When used in combination with the above thrombolytic agents, the compounds of the present invention may be administered prior to, simultaneously with, or subsequent to the antithrombolytic agent. Mammals exposed to renal dialysis, blood oxygenation, cardiac catheterization and similar medical procedures as well as mammals fitted with certain prosthetic devices are also susceptible to thromboembolic disorders. Physiologic conditions, with or without known cause may also lead to thromboembolic disorders. Thus, the compounds described herein are useful in treating thromboembolic disorders in mammals. The compounds described herein may also be used as adjuncts to anticoagulant therapy, for example in combination with aspirin, heparin or warfarin and other anticoagulant agents. The application of the compounds described herein for these and related disorders will be apparent to those skilled in the art.

Platelet Inhibition Assays

The evaluation of inhibitors of the fibrinogen-platelet interaction is guided by *in vitro* receptor binding assays and *in vitro* platelet aggregation inhibition assays.

In-vitro biological activity of the compounds of Formula I was monitored using a modified fibrinogen-GP IIbIIIa ELISA based on the method of Nachman and Leung (*J. Clin. Invest.* (1982) 69, 263-269) which measures the inhibition of fibrinogen binding to purified human platelet GP IIbIIIa receptor. Human fibrinogen was prepared by the method of Lipinska, et al. (*J. Lab. Clin. Med.* (1974) 84, 509-516). Platelet GP IIbIIIa was prepared by the method of Fitzgerald, et al. (*Anal. Biochem.* (1985) 151, 169-177.

Microtiter plates are coated with fibrinogen (10 μg/ml) and then blocked with TACTS buffer containing 0.5% bovine serum albumin (BSA). (TACTS buffer contains 20mM Tris.HCl, pH 7.5, 0.02% sodium azide, 2 mM calcium chloride, 0.05% Tween 20, 150 mM sodium chloride.) The plate is washed with phosphate buffered saline (PBS) containing 0.01% Tween 20 and the sample to be determined added, followed by addition

of solubilized GP IIbIIIa receptor (40 µg/ml) in TACTS, 0.5% BSA. After incubation, the plate is washed and 1 µg/ml of murine anti-platelet monoclonal antibody AP3 (P. J. Newman et al. *Blood* (1985) 65, 227-232) is added. After another wash a goat anti-mouse IgG conjugated to horseradish peroxidase is added. A final wash is performed and developing reagent buffer (10 mg o-phenylenediamine dihydrochloride, 0.0212% hydrogen peroxide, 0.22 mM citrate, 50 mM phosphate, pH 5.0) is added and then incubated until color develops. The reaction is stopped with 1N sulfuric acid and the absorbance at 492 nm is recorded.

In addition to the GP IIbIIIa ELISA assay, platelet aggregation assays may be performed in human platelet rich plasma (PRP). Fifty milliliters of whole human blood (9 parts) is drawn on 3.6% sodium citrate (1 part) from a donor who has not taken aspirin or related medications for at least two weeks. The blood is centrifuged at 160 x g for 10 min at 22° C and then allowed to stand for 5 min after which the PRP is decanted. Platelet poor plasma (PPP) is isolated from the remaining blood after centrifugation at 2000 x g for 25 min. The platelet count of the PRP was adjusted to ca. 300,000 per microliter with PPP.

A 225 µL aliquot of PRP plus 25 µL of either a dilution of the test sample or a control (PBS) is incubated for 5 min in a Chrono-log Whole Blood Aggregometer at 25° C. An aggregating agent (collagen, 1 mg/ml; U46619, 100 ng/ml; or ADP, 8 µM) is added and the platelet aggregation recorded.

In the management of thromboembolic disorders the compounds of this invention may be utilized in compositions such as tablets, capsules or elixers for oral administration; suppositories for rectal administration; sterile solutions or suspensions for injectable administration, and the like. Animals in need of treatment using compounds of this invention can be administered dosages that will provide optimal efficacy. The dose and method of administration will vary from animal to animal and be dependent upon such factors as weight, diet, concurrent medication and other factors which those skilled in the medical arts will recognize.

Dosage Formulations

Dosage formulations of the cyclic polypeptides of the present invention are prepared for storage or administration by mixing the the cyclic polypeptide having the desired degree of purity with physiologically acceptable carriers, excipients, or stabilizers. Such materials are non-toxic to the recipients at the dosages and concentrations employed, and include buffers such as phosphate, citrate, acetate and other organic acid salts; antioxidants such as ascorbic acid; low molecular weight (less than about ten residues) peptides such as polyarginine, proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidinone; amino acids such as glycine, glutamic acid, aspartic acid, or arginine; monosaccharides, disaccharides, and other carbohydrates including cellulose or its derivatives, glucose, mannose, or dextrins; chelating agents such as EDTA; sugar alcohols such as mannitol or sobitol; counterions such as sodium and/or nonionic surfactants such as Tween, Pluronics or polyethyleneglycol.

Dosage formulations of the cyclic polypeptides of the present invention to be used for therapeutic administration must be sterile. Sterility is readily accomplished by filtration through sterile filtration membranes such as 0.2 microne membranes. Cyclic polypeptide formulations ordinarily will be stored in lyophilized form or as an aqueous solution. The pH of the cyclic polypeptide preparations typically will be between 3 and 11, more preferably from 5 to 9 and most preferably from 7 to 8. It will be understood that use of certain of the foregoing excipients, carriers, or stabilizers will result in the formation of cyclic polypeptide salts. While the preferred route of administration is by hypodermic injection needle, other methods of administration are also anticipated

such as suppositories, aerosols, oral dosage formulations and topical formulations such as ointments, drops and dermal patches.

Therapeutic cyclic polypeptide formulations generally are placed into a container having a sterile access port, for example, an intravenous solution bag or vial having a stopper pierceable by hypodermic injection needle.

Therapeutically effective dosages may be determined by either *in vitro* or *in vivo* methods. One method of evaluating therapeutically effective dosages is illustrated in Example 23 where the cyclic polypeptide cyclo-S-acetyl-Gly-Arg-Gly-Asp-Cys-OH was determined to have a 50% inhibitory concentration (IC_{50}) of 5 nM when inhibiting fibrinogen binding to the GP IIbIIIa platelet receptor. Similarly, in a platelet aggregation assay in Example 24 using the same cyclic peptide, the IC_{50} was found to be 8.5 μM. Based upon such *in vitro* assay techniques, a therapeutically effective dosage range may be determined. For each particular cyclic polypeptide of the present invention, individual determinations may be made to determine the optimal dosage required. The range of therapeutically effective dosages will naturally be influenced by the route of administration. For injection by hypodermic needle it may be assumed the dosage is delivered into the body's fluids. For other routes of administration, the absorption efficiency must be individually determined for each cyclic polypeptide by methods well known in pharmacology.

The range of therapeutic dosages is from about 0.001 nM to 1.0 mM, more preferably from 0.1 nM to 100 μM, and most preferably from 1.0 nM to 50 μM.

Typical formulation of compounds of Formula I as pharmaceutical compositions are discussed below.

About 0.5 to 500 mg of a compound or mixture of compounds of Formula I, as the free acid or base form or as a pharmaceutically acceptable salt, is compounded with a physiologically acceptable vehicle, carrier, excipient, binder, preservative, stabilizer, flavor, etc., as called for by accepted pharmaceutical practice. The amount of active ingredient in these compositions is such that a suitable dosage in the range indicated is obtained.

Typical adjuvants which may be incorporated into tablets, capsules and the like are a binder such as acacia, corn starch or gelatin; an excipient such as microcrystalline cellulose; a disintegrating agent like corn starch or alginic acid; a lubricant such as magnesium stearate; a sweetening agent such as sucrose or lactose; a flavoring agent such as peppermint, wintergreen or cherry. When the dosage form is a capsule, in addition to the above materials it may also contain a liquid carrier such as a fatty oil. Other materials of various types may be used as coatings or as modifiers of the physical form of the dosage unit. A syrup or elixer may contain the active compound, a sweetener such as sucrose, preservatives like propyl paraben, a coloring agent and a flavoring agent such as cherry. Sterile compositions for injection can be formulated according to conventional pharmaceutical practice. For example, dissolution or suspension of the active compound in a vehicle such as water or naturally occurring vegetable oil like sesame, peanut, or cottonseed oil or a synthetic fatty vehicle like ethyl oleate or the like may be desired. Buffers, preservatives, antioxidants and the like can be incorporated according to accepted pharmaceutical practice.

EXAMPLES

In the following Examples, common α-amino acids may be described by the standard three letter amino acid code when referring to intermediates and final products. By common α-amino acids is meant those amino acids incorporated into proteins under mRNA direction. Standard abbreviations are listed in **The Merck Index**, 10th Edition, pp Misc-2 - Misc-3. Unless otherwise designated the common α-amino acids have the natural or "L"-

configuration at the alpha carbon atom. If the code is preceded by a "D" this signifies the opposite enantiomer of the common α-amino acid. Modified or unusual α-amino acids such as norleucine (Nle) and ornithine (Orn) are designated as described in U.S. Patent and Trademark Office Official Gazette 1114TMOG, May 15, 1990. If the product or intermediate name is preceded by "cyclo" this shall be taken to mean that the peptide has been cyclized, e.g. compounds of Formula I or V.

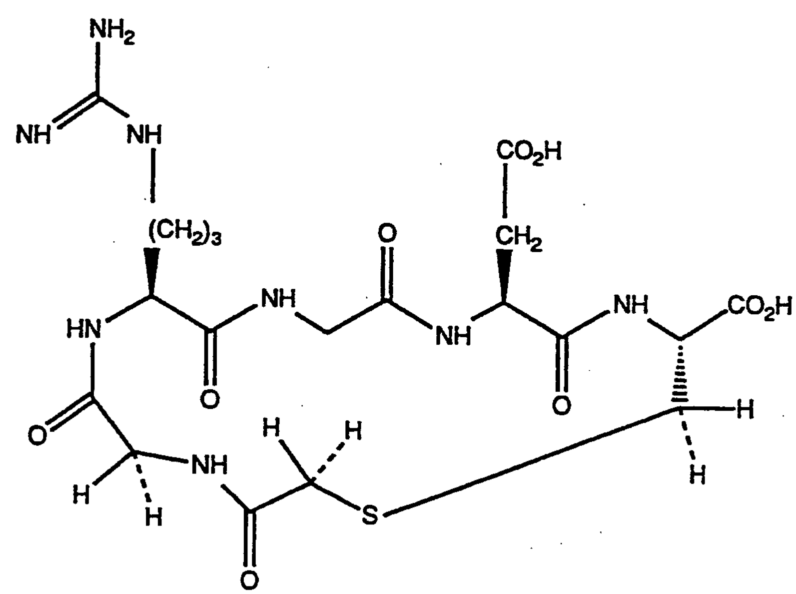
Example 1

Bromoacetyl-Gly-Arg-Gly-Asp-Cys-OH

The title compound is prepared in protected form by standard solid phase peptide synthesis on 2% cross-linked polystyrene resin (Merrifield resin). Treatment of the resin bound intermediate with liquid hydrogen fluoride induces concomitant cleavage of the protecting groups from the title compound as well as cleavage of the peptide from the resin. The crude peptide is purified by reverse phase high performance liquid chromatography (HPLC) using a 4.6 mm x 250 mm column containing 10 micron, 300 Angstrom pore size C-18 packing. The elution of the column is with an acetontrile/0.1% aqueous trifluoroacetic acid gradient going from 0% - 40% acetonitrile linearly over 80 minutes. The title compound elutes at 14 minutes. FAB mass spectrum: calc. 627; obs. 628 (M+1).

Example 2

Cyclo-S-acetyl-Gly-Arg-Gly-Asp-Cys-OH



The compound prepared in Example 1 is dissolved in deionized water (1mg/ml) and the pH of the solution is adjusted to 7.0-8.5 with ammonium hydroxide. After stirring for 4 hr at ambient temperature the reaction solution is acidified to pH 3.0 - 3.5 with trifluoroacetic acid and then lyophilized. The resulting crude product is purified by HPLC using the conditions described in Example 1. The desired title compound elutes after 11 minutes. FAB mass spectrum: calc. 546; obs. 547 (M+1). Amino acid analysis: carboxymethyl-cys, 0.96; Asp, 1.04; Gly, 2.12; Arg, 0.94. 1H NMR(300 MHz, D_2O, pH 7): 4.75, m; 4.3-4.4, m; 4.15, d; 4.0, ab q; 3.9, d; 3.4, ab q; 3.2, t; 2.95-3.15, m; 2.7, dq; 1.75-2.05, m; 1.55-1.7,m.

Example 3

Cyclo-S-acetyl-Gly-Arg-Gly-Asp-Cys-OH

Bromoacetyl-Gly-Arg(g-2,2,5,7,8-pentamethylchroman-6-sulfonyl)-Gly-Asp(beta-t-butyl)-Cys(S-triphenylmethyl)-O-(polymer resin) is prepared using standard solid phase peptide synthesis utilizing fluorenylmethoxycarbonyl (FMOC) protecting group chemistry on a p-alkoxybenzyl alcohol resin. Repeated treatment of the resin bound peptide with a 1% solution of trifluoroacetic acid in dichloromethane results in cleavage of the S-triphenylmethyl group as evidenced by the bright yellow of the solution. Treatment is continued until dissipation of the yellow color (ca. 1.5 L of the cleavage solution is required per gram of resin bound peptide.) After complete cleavage of the S-triphenylmethyl group, the resin bound peptide is washed several times with a 5% solution of N-methylmorpholine in N,N-dimethylacetamide and then shaken in pure N,N-dimethylacetamide for 12 hr to complete the cyclization. Treatment of the cyclized resin bound peptide with trifluoroacetic acid containing (v/v) 1% phenol, 1% anisole and 1% ethanedithiol effects concomitant cleavage of the remaining protective groups and cleavage of the desired product from the resin. Purification of the crude product as described in Example 2 affords the title compound identical to that described above.

Example 4

Synthesis of Compounds of Formula VII

Using the methods described in Examples 1 and 2, the compounds listed in Table I may be prepared. The compounds are depicted by Formula VII wherein m = 1 and n = 3, R_1 and R_9 are OH, R_{14} is hydrogen and X is S. Crude products are purified using HPLC as described in Example 2.

The following amino acid derivatives may be used in place of Boc-Gly for coupling to the alpha-amine group of Arg to obtain the substituents R_2 and R_3 shown in Table I. When Boc-Glycine is used, R_2 and R_3 are both hydrogen (H).

Amino Acid Derivative	R_2	R_3
Boc-L-Ala	methyl	H
Boc-D-Ala	H	methyl
Boc-L-Val	2-propyl	H
Boc-D-Val	H	2-propyl
Boc-D-Thr	H	1-hydroxy-1-ethyl
Boc-L-Thr	1-hydroxy-1-ethyl	H
Boc-D-Asn	H	carboxamidomethyl
Boc-L-Asn	carboxamidomethyl	H
Boc-D-Gln	H	2-carboxamidoethyl
Boc-L-Gln	2-carboxamidoethyl	H
Boc-D-(O-2-bromobenzyloxy-carbonyl)Tyr	H	4-hydroxybenzyl
Boc-L-(O-2-bromobenzyloxy-carbonyl)Tyr	4-hydroxybenzyl	H

Boc-D-Phe	H	benzyl
Boc-L-Phe	benzyl	H
Boc-D-Leu	H	2-methyl-1-propyl
Boc-L-Leu	2-methyl-1-propyl	H
Boc-D-Met	H	2-methylthioethyl
Boc-L-Met	2-methylthiomethyl	H
Boc-D-Nle	H	1-butyl
Boc-L-Nle	1-butyl	H
Boc-D-Ile	H	2-butyl
Boc-L-Ile	2-butyl	H
Boc-D-Asp(β-O-cyclohexyl)	H	carboxymethyl
Boc-L-Asp(β-O-cyclohexyl)	carboxymethyl	H
Boc-D-Glu(γ-O-cyclohexyl)	H	2-carboxyethyl
Boc-L-Glu(γ-O-cyclohexyl)	2-carboxyethyl	H
Boc-D-(O-benzyl)Ser	H	hydroxymethyl
Boc-L-(O-benzyl)Ser	hydroxymethyl	H
Boc-D-(N^{im}-benzyloxymethyl)His	H	4-imidazolylmethyl
Boc-L-(N^{im}-benzyloxymethyl)His	4-imidazolylmethyl	H
Boc-D-Trp	H	3-indolylmethyl
Boc-L-Trp	3-indolylmethyl	H
Boc-D-(N^{ε}-2-chlorocarbobenzyl-oxy)Lys	H	4-amino-1-butyl
Boc-L-(N^{ε}-2-chlorocarbobenzyl-oxy)Lys	4-amino-1-butyl	H
Boc-D-(N^{δ}-carbobenzyoxy)Orn	H	3-amino-1-propyl
Boc-L-(N^{δ}-carbobenzyoxy)Orn	3-amino-1-propyl	H
Boc-D-(O-benzyl)Thr	H	1-hydoxy-1-ethyl
Boc-L-(O-benzyl)Thr	1-hydoxy-1-ethyl	H

Boc-L-Pro gives $R_2 + R_4 = CH_2CH_2CH_2$

Boc-D-Pro gives $R_3 + R_4 = CH_2CH_2CH_2$

The substituted bromoacetic acids listed below may be used in place of bromoacetic acid in Example 1 and in combination with the amino acid derivatives listed above to provide the compounds shown in Table 1 with variable substituents at R_5, R_6. When bromoacetic acid is used in combination with the amino acid derivatives listed above R_5 and R_6 are hydrogen (H).

1-naphthyl-α-bromoacetic acid
2-naphthyl-α-bromoacetic acid
phenyl-α-bromoacetic acid

2-trifluromethylphenyl-α-bromoacetic acid

3-trifluromethylphenyl-α-bromoacetic acid

4-trifluromethylphenyl-α-bromoacetic acid

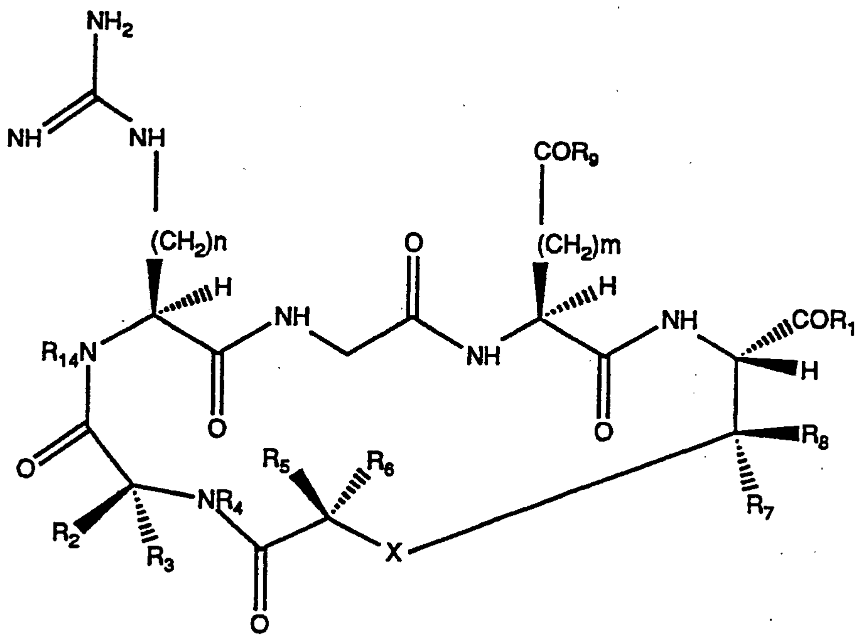
4-biphenyl-α-bromoacetic acid

2-bromopropionic acid

2-bromobutyric acid

2-bromopentanoic acid

L-Pennicillamine may be substituted for L-cysteine in Example 1 to produce compounds in Table 1 where R_7 and R_8 are methyl.



VII

TABLE I

Selected compounds of Formula VII

R_2	R_3	R_4	R_5, R_6	R_7	R_8	MW[1] (calc)	MW[2] (obs)	RT[3]
H	(4)	(4)	H, H	H	H	586.2	587	16
H	1-hydroxy-1-ethyl	H	H, H	H	H	590.2	591.0	9
H	H	H	H, 1-naphthyl	H	H	672.2	673.0	40
H	1-hydroxy-1-ethyl	H	H, phenyl	H	H	666.2	667.2	26

H	H	H	H, 4-biphenyl	H	H	698.4	699.0	50
H	4-hydroxy-benzyl	H	H, H	H	H	652.2	653.2	20
H	H	H	H, phenyl	H	H	622.2	623.1	18
H	H	H	H, H	CH_3	CH_3	574.2	574.9	14
H	2-propyl	H	H, H	H	H	588.2	588.9	18
H	methyl	H	H, H	H	H	560.2	560.9	10
H	carboxamido methyl	H	H, H	H	H	603.2	604.1	11
H	H	H	H, 2-tri-fluoromethyl-phenyl	H	H	690.3	691.0	38
H	4-imidazolyl-methyl	H	H, H	H	H	626.2	627.0	8
H	2-methyl-1-propyl	H	H, H	H	H	602.3	602.9	23
H	4-hydroxy-benzyl	H	H, phenyl	H	H	728.2	729.0	38
H	H	H	H, 4-biphenyl	H	H	698.4	699.0	52
H	H	H	H, 1-naphthyl	H	H	672.3	673.0	44
H	2-methyl-thioethyl	H	H, H	H	H	620.2	621.0	18
H	hydroxy-methyl	H	H, H	H	H	576.2	577.0	8
H	3-indolyl-methyl	H	H, H	H	H	675.3	676.1	28
H	carboxamido ethyl	H	H, H	H	H	617.2	617.9	9
H	H	H	H, 4-tri-fluoromethyl phenyl	H	H	690.3	691.0	44
H	H	H	H, 2-naphthyl	H	H	672.2	673.0	39
H	H	H	H, propyl	H	H	588.2	589.0	24

H	3-amino-1-propyl	H	H, H	H	H	603.2	603.9	6
H	H	H	H, 3-tri-fluoromethyl phenyl	H	H	690.3	691.0	42
H	H	H	H, ethyl	H	H	574.2	575.0	18
H	2-butyl	H	H, H	H	H	602.3	603.0	22
4-imidazolyl-methyl	H	H	H, H	H	H	626.2	627.1	14
benzyl	H	H	H, H	H	H	636.2	637.0	25
H	1-hydroxy-1-ethyl	H	H, phenyl	H	H	666.2	667.2	28
H	4-hydroxy-benzyl	H	H, H	CH_3	CH_3	680.2	681.0	21
H	4-amino-1-butyl	H	H, H	H	H	617.3	618.0	8
CH_3	H	H	H, H	H	H	560.2	561.0	13
(5)	H	(5)	H, H	H	H	586.2	587.0	17
H	H	H	H, 2-naphthyl	H	H	672.3	673.0	42
1-hydroxy-1-ethyl	H	H	H, H	H	H	590.2	591.0	12
H	H	H	H, phenyl	H	H	622.2	623.1	16
H	2-carboxy-1-ethyl	H	H, H	H	H	618.2	619.0	11
1-butyl	H	H	H, H	H	H	602.2	---	---
2-propyl	H	H	H, H	H	H	588.2	589.0	19
2-butyl	H	H	H, H	H	H	602.3	602.8	24
4-hydroxy-benzyl	H	H	H,H	H	H	652.2	653.2	23
H	H	H	H, ethyl	H	H	574.2	575.0	16
4-amino-1-butyl	H	H	H, H	H	H	617.3	618.0	10
H	H	H	H, 1-propyl	H	H	588.2	589.0	22
hydroxy-methyl	H	H	H, H	H	H	576.2	576.1	10

H	H	H	H, 2-trifluoromethyl phenyl	H	H	690.3	691.0	36
H	carboxymethyl	H	H, H	H	H	604.2	605.1	12
2-methylthioethyl	H	H	H, H	H	H	620.2	620.8	20
3-indolylmethyl	H	H	H, H	H	H	675.3	676.0	32
3-amino-1-propyl	H	H	H, H	H	H	603.2	604.2	5
carboxamido methyl	H	H	H, H	H	H	603.2	603.9	9
benzyl	H	H	H, H	H	H	636.2	637.0	28
H	H	H	H, 3-trifluoromethyl phenyl	H	H	690.3	691.0	40
2-methyl-1-propyl	H	H	H, H	H	H	602.3	602.8	24
H	H	H	H, CH_3	H	H	560.2	561.0	14
H	H	H	H, pentafluorophenyl	H	H	712.3	712.9	36
2-carboxamidoethyl	H	H	H, H	H	H	617.2	618.0	10
H	H	H	H, 4-trifluoromethyl phenyl	H	H	690.3	691.0	42
2-carboxyethyl	H	H	H, H	H	H	618.2	618.8	12
carboxymethyl	H	H	H, H	H	H	604.2	604.8	10

Notes

(1) Calculated molecular weight of the peptide.

(2) Observed molecular weight for M+1, where M is the molecular ion, determined by FAB mass spectrometry.

(3) Observed chromatographic retention time of the peptide in minutes determined using the conditions given in Example 2.

(4) $R_3 + R_4 = CH_2CH_2CH_2$.

(5) $R_2 + R_4 = CH_2CH_2CH_2$.

Example 5

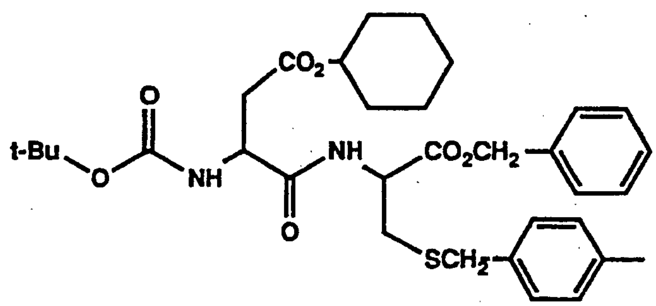
N-(N-t-Butoxycarbonyl(Ng-p-toluenesulfonyl)arginyl) glycine

N-t-Butoxycarbonyl-Ng-p-toluenesulfonylarginine (10 mmoles), benzotriazol-1-yloxytris(dimethylamino)phosphonium hexafluorophosphate (BOP, 10 mmoles), ethyl glycinate hydrochloride (13 mmoles) and N-methylmorpholine (50 mmoles) are stirred in a mixture of dichloromethane and N,N-dimethylacetamide (1:1, 50 ml) for 2 hr at room temperature. At the end of the reaction time the mixture is acidified with acetic acid, concentrated in vacuo, and the residue taken up in ethyl acetate. The resulting solution is washed with 10% citric acid (3 times), water (2 times), saturated sodium bicarbonate (3 times), water (2 times) and brine (2 times). The organic solution is dried over magnesium sulfate, filtered, dried and concentrated to afford the crude ethyl ester of the title compound. Crystalline material can be obtained from ethyl acetate-hexane mixtures.

The dipeptide ethyl ester (10 mmoles) is treated with 10.5 mmoles of sodium hydroxide in ethanol and water (4:1, 50 ml) for 1 hr. at room temperature. Water (100 ml) is added and the mixture concentrated to ca 50 ml, acidified with citric acid, and extracted with ethyl acetate. After drying the extract over magnesium sulfate, concentration affords the desired title compound. It may be recrystallized from ethyl acetate-hexane mixtures.

Example 6

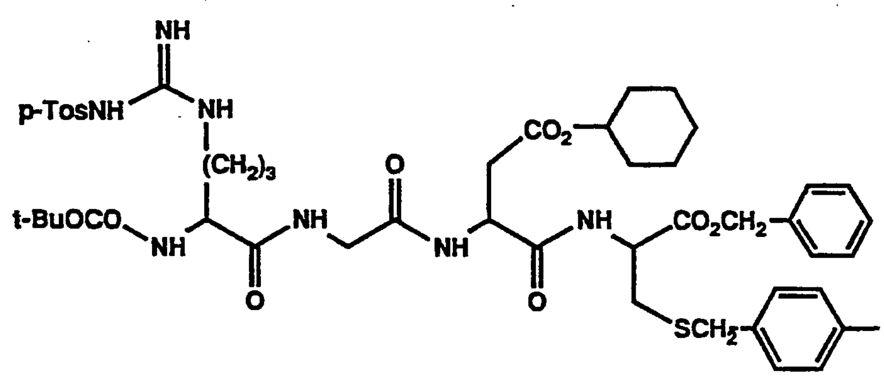
N-(N-t-butoxycarbonyl(beta-cyclohexyl ester)aspartyl) cysteine (S-4-methylbenzyl) benzyl ester



N-t-Butoxycarbonyl(beta-cyclohexyl ester)aspartic acid (10 mmole), BOP reagent (10 mmole), and N-methylmorpholine (15 mmole) are stirred in N,N-dimethylacetamide (50 ml) at room temperature for 10 min. To the resulting solution is added a solution of cysteine(S-4-methylbenzyl) benzyl ester trifluoroacetate (10 mmole) and N-methylmorpholine (15 mmole) in N,N-dimethylacetamide (15 ml). The resulting reaction mixture is stirred overnight at room temperature. The solvent is then removed in vacuo and the reaction residue worked up as described in Example 5 to afford the desired title compound.

Example 7

N-(N-(N-(N-t-Butoxycarbonyl(N^g-p-toluenesulfonyl)arginyl) glycinyl)aspartyl(beta-cyclohexyl ester))cysteine(S-4-methylbenzyl) benzyl ester

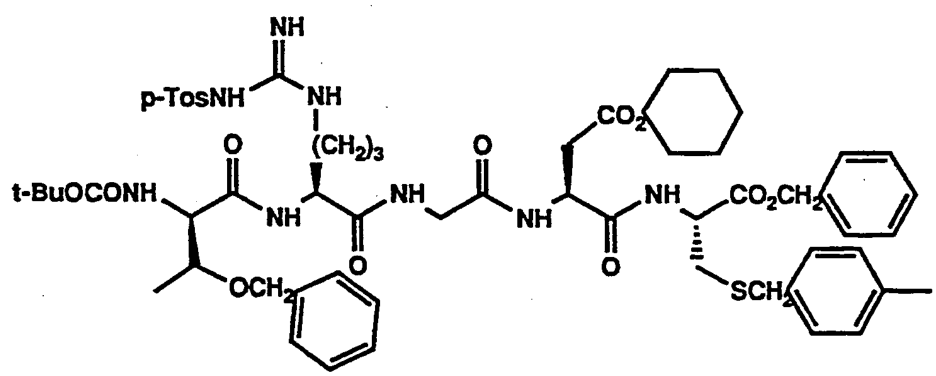


N-(N-t-butoxycarbonyl(beta-cyclohexyl ester)aspartyl) cysteine (S-4-methylbenzyl) benzyl ester from Example 6 (10 mmole) is treated with trifluoroacetic acid in dichloromethane (1:1, 200 ml) for 40 min at room temperature and then concentrated. The resulting solid is washed with ether and dried.

The above solid (10 mmole), BOP reagent (10 mmole), N-methylmorpholine (40 mmole) and N-(N-t-Butoxycarbonyl(N^g-p-toluenesulfonyl)arginyl)glycine from Example 22 (10 mmole) are stirred in N,N-dimethylacetamide (50 ml) for 4 hr. After the reaction the solution is concentrated and the residue taken up in ethyl acetate and worked up as described in Example 5. The crude product thus obtained is crystallized from chloroform-hexane.

Example 8

N-(N-(N-(N-(N-t-Butoxycarbonyl(O-benzyl)-D-threonyl)(N^g-p-toluenesulfonyl)arginyl) glycinyl)aspartyl(beta-cyclohexyl ester))cysteine(S-4-methylbenzyl) benzyl ester

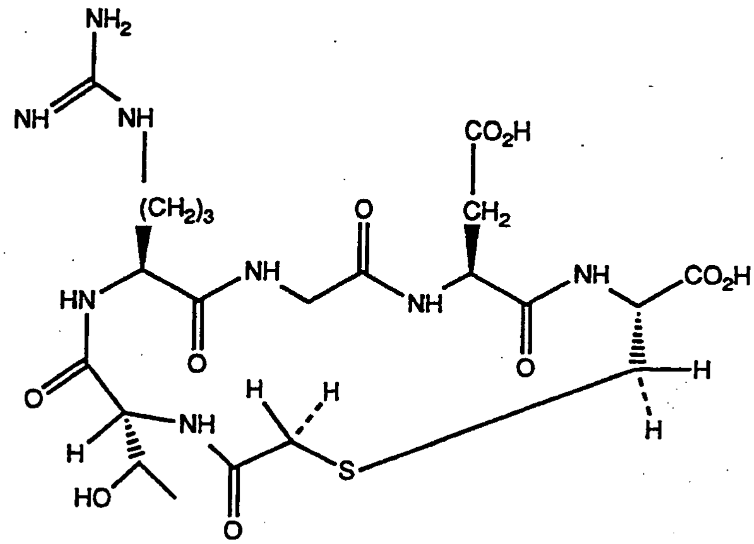


The t-butoxycarbonyl group is removed from N-(N-(N-(N-t-butoxycarbonyl(N^g-p-toluenesulfonyl)arginyl) glycinyl)aspartyl(beta-cyclohexyl ester))cysteine(S-4-methylbenzyl) benzyl ester using the procedure described in Example 7 to afford the trifluoroacetate salt of the tetrapeptide.

The above salt is coupled with N-t-butoxycarbonyl(O-benzyl)-D-threonine using the procedure described in Example 7. The desired title compound is isolated after workup as described above.

Example 9

Cyclo-S-acetyl-(D-Thr)-Arg-Gly-Asp-Cys-OH



The t-butoxycarbonyl group is removed from N-(N-(N-(N-(N-t-Butoxycarbonyl(O-benzyl)-D-threonyl)(N^g-p-toluenesulfonyl)arginyl) glycinyl)aspartyl(beta-cyclohexyl ester))cysteine(S-4-methylbenzyl) benzyl ester using the procedure described in Example 7 to afford the trifluoroacetate salt of the pentapeptide.

Bromoacetic acid (4 mmole) and dicyclohexylcarbodiimide (2 mmole) are stirred together in 5 ml of dichloromethane for 10 min. The solution is filtered and added to the pentapeptide trifluoroacetate prepared above (1 mmole) and N-methylmorpholine (3 mmole) in N,N-dimethylacetamide (5 ml). After stirring for 1 hr at room temperature the reaction is concentrated in vacuo and the residue taken up in ethyl acetate and worked up as described in Example 5. The residue isolated from the ethyl acetate solution is treated directly with a mixture of hydrogen fluoride, anisole and methylethyl sulfide (90:5:5) at 0 C for 1 hr. After removal of the hydrogen fluoride the crude material is dissolved in 10% acetic acid and lyophilized. The isolated powder is then dissolved in water at a concentration of 1 mg/ml and the pH adjusted to 7.3 with ammonium hydroxide. After stirring at ambient temperature for 4 hr the reaction solution is loaded onto a DEAE 52 cellulose column equilibrated in 5 mM ammonium acetate at pH 7.3. The desired title compound is eluted with 45 mM ammonium acetate at pH 7.3. The fractions containing the desired cyclized peptide are desalted immediately by acidification to pH 4 with acetic acid and chromatography over a C-18 reverse phase column. The loaded column is first washed with 0.1% aqueous trifluoroacetic acid followed by linear gradient with 0.1% trifluoroacetic acid in acetonitrile. The purified peptide, after lyophilization, is identical with the product described in Example 7.

Example 10

Method for Preparation of Other Compounds of Formula VII

Using the methods described in Examples 5-9, the replacement amino acids shown below may be used in place of D-threonine in Example 8 and the replacement bromo acids listed below may be used in place of bromoacetic acid in Example 9 to produce the compounds listed in Table 2. In this example, R_1 and R_9 are OH, R_{14}

is hydrogen, and X is S. The benzyl ester of S-(4-methylbenzyl)-L-pennicillamine may be used in place of the corresponding cysteine derivative in Example 5 to produce compounds in which R_7 and R_8 are methyl.

Amino Acid Derivative	R_2	R_3
Boc-L-Ala	methyl	H
Boc-D-Ala	H	methyl
Boc-L-Val	2-propyl	H
Boc-D-Val	H	2-propyl
Boc-D-Thr	H	1-hydroxy-1-ethyl
Boc-L-Thr	1-hydroxy-1-ethyl	H
Boc-D-Asn	H	carboxamidomethyl
Boc-L-Asn	carboxamidomethyl	H
Boc-D-Gln	H	2-carboxamidoethyl
Boc-L-Gln	2-carboxamidoethyl	H
Boc-D-(O-2-bromobenzyloxy-carbonyl)Tyr	H	4-hydroxybenzyl
Boc-L-(O-2-bromobenzyloxy-carbonyl)Tyr	4-hydroxybenzyl	H
Boc-D-Phe	H	benzyl
Boc-L-Phe	benzyl	H
Boc-D-Leu	H	2-methyl-1-propyl
Boc-L-Leu	2-methyl-1-propyl	H
Boc-D-Met	H	2-methylthioethyl
Boc-L-Met	2-methylthioethyl	H
Boc-D-Nle	H	1-butyl
Boc-L-Nle	1-butyl	H
Boc-D-Ile	H	2-butyl
Boc-L-Ile	2-butyl	H
Boc-D-Asp(β-O-cyclohexyl)	H	carboxymethyl
Boc-L-Asp(β-O-cyclohexyl)	carboxymethyl	H
Boc-D-Glu(γ-O-cyclohexyl)	H	2-carboxyethyl
Boc-L-Glu(γ-O-cyclohexyl)	2-carboxyethyl	H
Boc-D-(O-benzyl)Ser	H	hydroxymethyl
Boc-L-(O-benzyl)Ser	hydroxymethyl	H
Boc-D-(N^{im}-benzyloxymethyl)His	H	4-imidazolylmethyl
Boc-L-(N^{im}-benzyloxymethyl)His	4-imidazolylmethyl	H
Boc-D-Trp	H	3-indolylmethyl
Boc-L-Trp	3-indolylmethyl	H

Boc-D-(N^{ϵ}-2-chlorocarbobenzyl-oxy)Lys	H	4-amino-1-butyl
Boc-L-(N^{ϵ}-2-chlorocarbobenzyl-oxy)Lys	4-amino-1-butyl	H
Boc-D-(N^{δ}-carbobenzyoxy)Orn	H	3-amino-1-propyl
Boc-L-(N^{δ}-carbobenzyoxy)Orn	3-amino-1-propyl	H
Boc-D-(O-benzyl)Thr	H	1-hydoxy-1-ethyl
Boc-Gly	H	H

Boc-L-Pro gives $R_2 + R_4 = CH_2CH_2CH_2$

Boc-D-Pro gives $R_3 + R_4 = CH_2CH_2CH_2$

Replacement bromo acids

1-naphthyl-α-bromoacetic acid
2-naphthyl-α-bromoacetic acid
phenyl-α-bromoacetic acid
2-trifluromethylphenyl-α-bromoacetic acid
3-trifluromethylphenyl-α-bromoacetic acid
4-trifluromethylphenyl-α-bromoacetic acid
4-biphenyl-α-bromoacetic acid
2-bromopropionic acid
2-bromobutyric acid
2-bromopentanoic acid

When bromoacetic acid is used in combination with the amino acid derivatives listed above R_5 and R_6 are hydrogen (H).

Table 2

Other Selected Compounds of Formula VII

R_2	R_3	R_4	R_5, R_6	R_7	R_8	MW[1] (calc)	MW[2] (obs)	RT[3]
H	(4)	(4)	H, H	H	H	586.2	587	16
H	1-hydroxy-1-ethyl	H	H, H	H	H	590.2	591.0	9
H	4-hydroxy-benzyl	H	H, H	H	H	652.2	653.2	20
H	H	H	H, H	CH_3	CH_3	574.2	574.9	14
H	2-propyl	H	H, H	H	H	588.2	588.9	18
H	methyl	H	H, H	H	H	560.2	560.9	10

H	carboxamido methyl	H	H, H	H	H	603.2	604.1	11
H	4-imidazolyl-methyl	H	H, H	H	H	626.2	627.0	8
H	2-methyl-1-propyl	H	H, H	H	H	602.3	602.9	23
H	2-methyl-thioethyl	H	H, H	H	H	620.2	621.0	18
H	hydroxy-methyl	H	H, H	H	H	576.2	577.0	8
H	3-indolyl-methyl	H	H, H	H	H	675.3	676.1	28
H	carboxamido ethyl	H	H, H	H	H	617.2	617.9	9
H	H	H	H, H	H	H	546.2	547.0	10
H	3-amino-1-propyl	H	H, H	H	H	603.2	603.9	6
H	2-butyl	H	H, H	H	H	602.3	603.0	22
4-imidazolyl-methyl	H	H	H, H	H	H	626.2	627.1	14
benzyl	H	H	H, H	H	H	636.2	637.0	25
H	4-hydroxy-benzyl	H	H, H	CH_3	CH_3	680.2	681.0	21
H	4-amino-1-butyl	H	H, H	H	H	617.3	618.0	8
CH_3	H	H	H, H	H	H	560.2	561.0	13
(5)	H	(5)	H, H	H	H	586.2	587.0	17
1-hydroxy-1-ethyl	H	H	H, H	H	H	590.2	591.0	12
H	2-carboxy-1-ethyl	H	H, H	H	H	618.2	619.0	11
1-butyl	H	H	H, H	H	H	602.2	---	---
2-propyl	H	H	H, H	H	H	588.2	589.0	19
2-butyl	H	H	H, H	H	H	602.3	602.8	24

4-hydroxy-benzyl	H	H	H,H	H	H	652.2	653.2	23
4-amino-1-butyl	H	H	H, H	H	H	617.3	618.0	10
hydroxy-methyl	H	H	H, H	H	H	576.2	576.1	10
H	carboxy-methyl	H	H, H	H	H	604.2	605.1	12
2-methyl-thioethyl	H	H	H, H	H	H	620.2	620.8	20
3-indolyl-methyl	H	H	H, H	H	H	675.3	676.0	32
3-amino-1-propyl	H	H	H, H	H	H	603.2	604.2	5
carboxamido methyl	H	H	H, H	H	H	603.2	603.9	9
benzyl	H	H	H, H	H	H	636.2	637.0	28
2-methyl-1-propyl	H	H	H, H	H	H	602.3	602.8	24
2-carbox-amidoethyl	H	H	H, H	H	H	617.2	618.0	10
2-carboxy-ethyl	H	H	H, H	H	H	618.2	618.8	12
carboxy-methyl	H	H	H, H	H	H	604.2	604.8	10
H	1-hydroxy-1-ethyl	H	H, phenyl	H	H	666.2	667.2	26(6)
H	1-hydroxy-1-ethyl	H	H, phenyl	H	H	666.2	667.2	28(7)
H	H	H	H, phenyl	H	H	622.2	623.1	16(6)
H	H	H	H, phenyl	H	H	622.2	623.1	18(7)
H	H	H	H, 4-biphenyl	H	H	698.4	699.0	50(6)
H	H	H	H, 4-biphenyl	H	H	698.4	699.0	52(7)
H	H	H	H, 1-naphthyl	H	H	672.2	673.0	39(6)
H	H	H	H, 1-naphthyl	H	H	672.2	673.0	40(7)
H	H	H	H, 2-naphthyl	H	H	672.3	673.0	42(6)

H	H	H	H, 2-naphthyl	H	H	672.3	673.0	44(7)
H	H	H	H, ethyl	H	H	574.2	575.0	16(6)
H	H	H	H, ethyl	H	H	574.2	575.0	18(7)
H	H	H	H, 1-propyl	H	H	588.2	589.0	22(6)
H	H	H	H, 1-propyl	H	H	588.2	589.0	24(7)
H	H	H	H, 2-trifluoromethyl-phenyl	H	H	690.3	691.0	36(6)
H	H	H	H, 2-trifluoromethyl phenyl	H	H	690.3	691.0	38(7)
H	H	H	H, 3-trifluoromethyl phenyl	H	H	690.3	691.0	40(6)
H	H	H	H, 3-trifluoromethyl phenyl	H	H	690.3	691.0	42(7)
H	H	H	H, 4-trifluoromethyl phenyl	H	H	690.3	691.0	42(6)
H	H	H	H, 4-trifluoromethyl phenyl	H	H	690.3	691.0	44(7)
H	H	H	H,CH_3	H	H	560.2	561.0	14
H	4-hydroxy-benzyl	H	H,phenyl	H	H	728.2	729.0	38

Notes

(1) Calculated molecular weight of the peptide.

(2) Observed molecular weight for M+1, where M is the molecular ion, based on FAB mass spectrometry.

(3) Observed chromatographic retention time of the peptide in minutes determined using the conditions given in Example 2.

(4) $R_3 + R_4 = CH_2CH_2CH_2$.

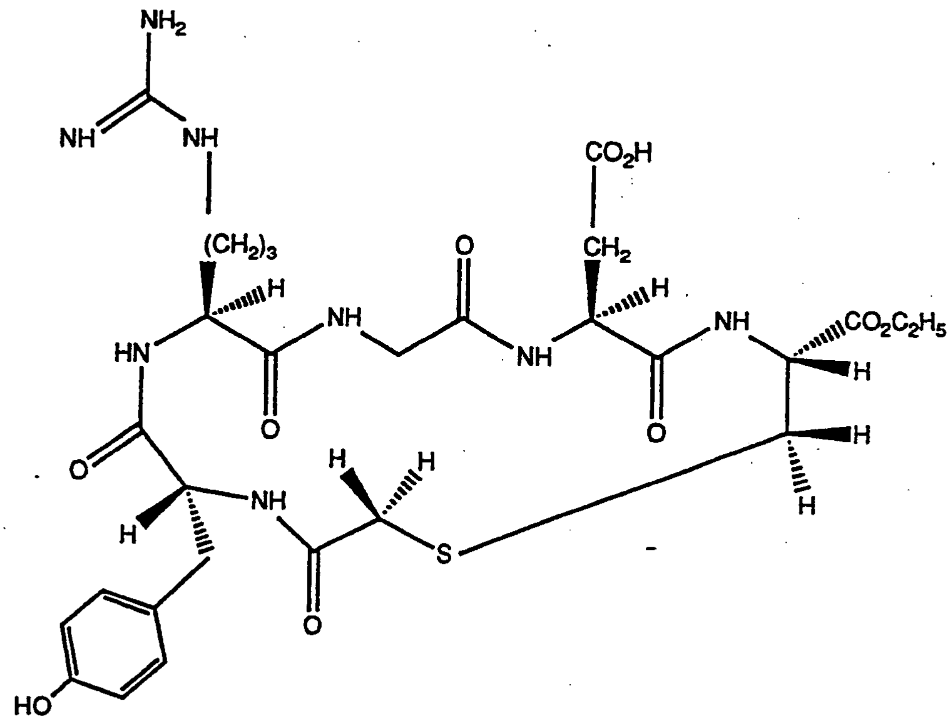
(5) $R_2 + R_4 = CH_2CH_2CH_2$.

(6) Isomer 1- first to elute from the chromatography column.

(7) Isomer 2- second to elute from the chromatography column.

Example 11

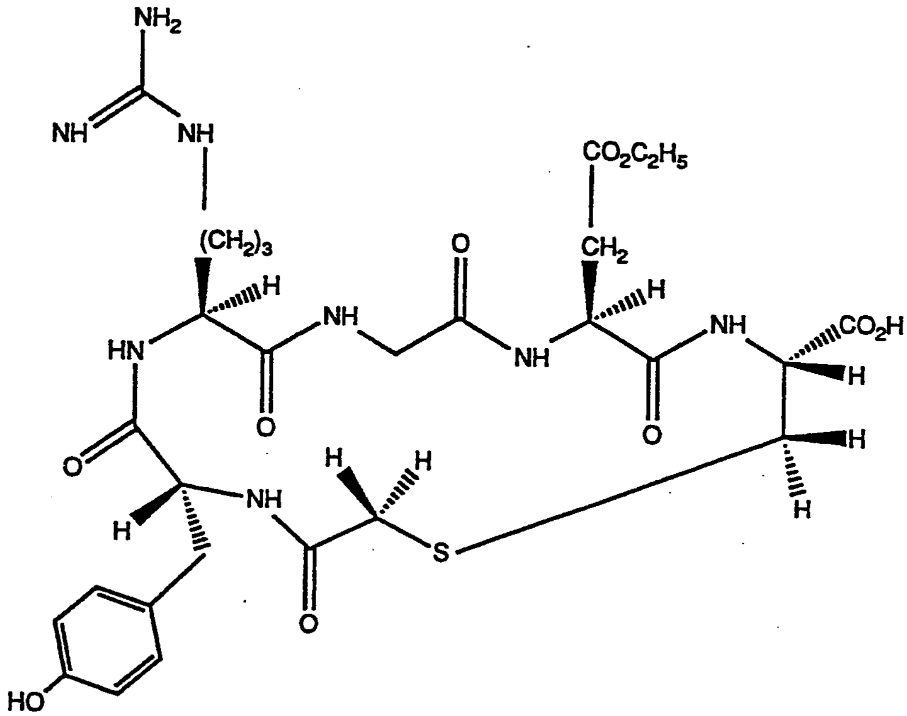
Cyclo-S-acetyl-(D-Tyr)-Arg-Gly-Asp-Cys-OC_2H_5



Using the procedures described in Examples 5-9 and replacing cysteine(S-4-methylbenzyl) benzyl ester trifluoroacetate with the corresponding ethyl ester the title compound shown above is obtained. HPLC retention time: 30 min. Molecular weight (calc.): 680.2. Found (FAB): 681.0 (M+1).

Example 12

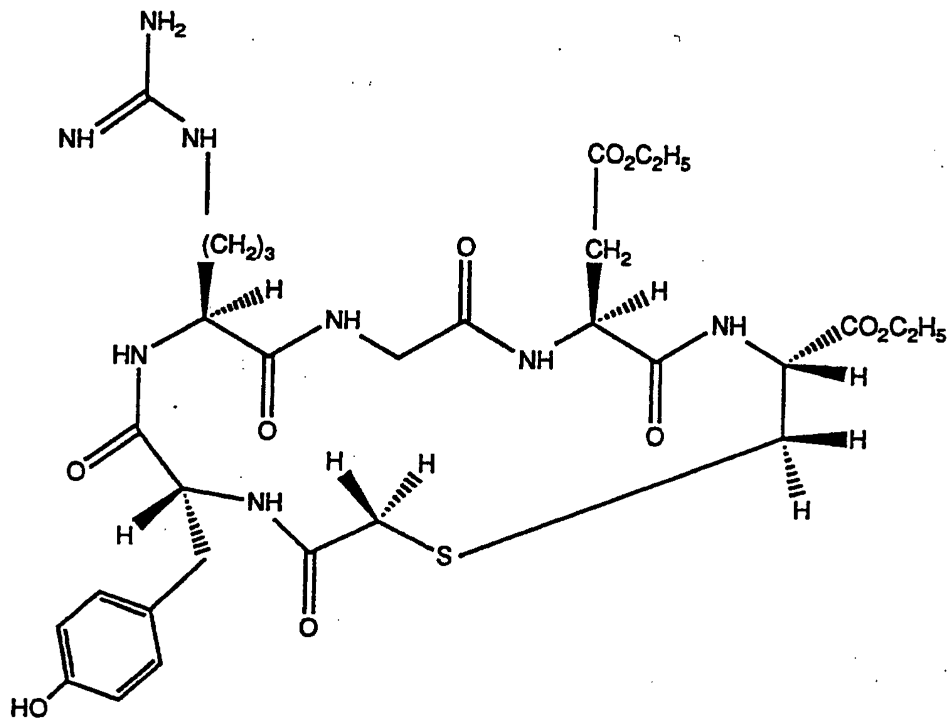
Cyclo-S-acetyl-(D-Tyr)-Arg-Gly-Asp(OC_2H_5)-Cys-OH



Using the procedures described in Examples 5-9 and replacing N-t-butoxycarbonyl(beta-cyclohexyl ester)aspartic acid with the corresponding beta-ethyl ester the title compound shown above is obtained. HPLC retention time: 26 min. Molecular weight (calc.): 680.2. Found (FAB): 681.2 (M+1).

Example 13

Cyclo-S-acetyl-(D-Tyr)-Arg-Gly-Asp(OC_2H_5)-Cys-OC_2H_5



Using the procedures described in Examples 5-9 and replacing cysteine(S-4-methylbenzyl) benzyl ester trifluoroacetate with the corresponding ethyl ester and N-t-butoxycarbonyl(beta-cyclohexyl ester)aspartic acid with the corresponding beta-ethyl ester the title compound shown above is obtained. HPLC retention time: 40 min. Molecular weight (calc.): 709.2. Found (FAB): 710.1 (M+1).

Example 14

Preparation of cyclic thioether peptide esters

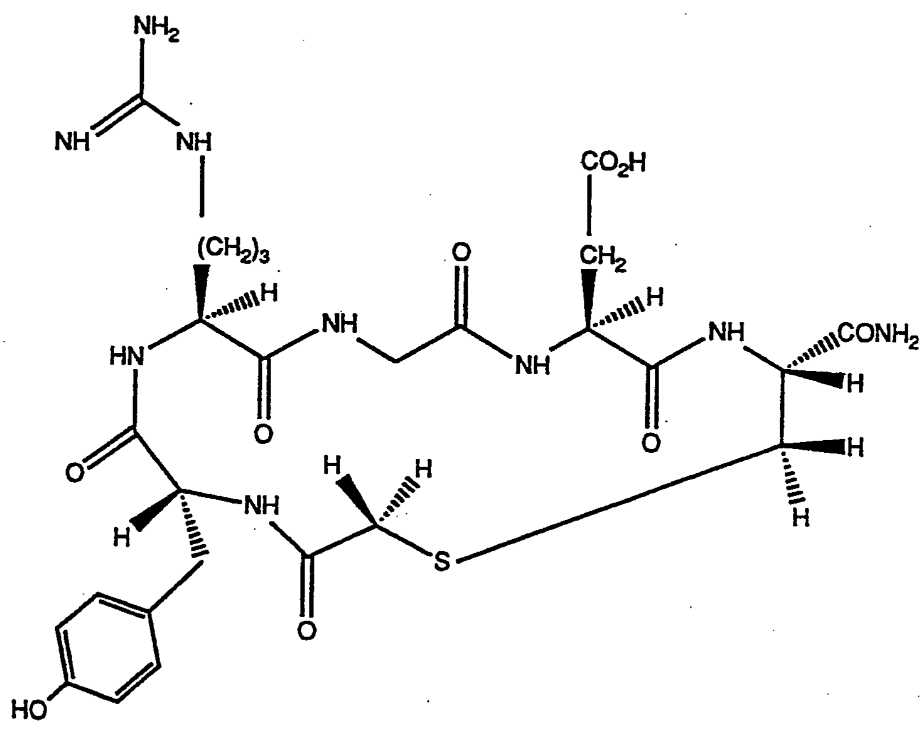
Using the methods described in Examples 11-13 above, the following cyclized peptide esters are analogously prepared.

Cyclo-S-acetyl-Gly-Arg-Gly-Asp-Cys-OC_2H_5
Cyclo-S-acetyl-Gly-Arg-Gly-Asp(OC_2H_5)-Cys-OH
Cyclo-S-acetyl-Gly-Arg-Gly-Asp(OC_2H_5)-Cys-OC_2H_5
Cyclo-S-acetyl-(D-Ala)-Arg-Gly-Asp-Cys-OC_2H_5
Cyclo-S-acetyl-(D-Ala)-Arg-Gly-Asp(OC_2H_5)-Cys-OH
Cyclo-S-acetyl-(D-Ala)-Arg-Gly-Asp(OC_2H_5)-Cys-OC_2H_5
Cyclo-S-acetyl-(D-Val)-Arg-Gly-Asp-Cys-OC_2H_5
Cyclo-S-acetyl-(D-Val)-Arg-Gly-Asp(OC_2H_5)-Cys-OH
Cyclo-S-acetyl-(D-Val)-Arg-Gly-Asp(OC_2H_5)-Cys-OC_2H_5
Cyclo-S-acetyl-(D-Leu)-Arg-Gly-Asp-Cys-OC_2H_5
Cyclo-S-acetyl-(D-Leu)-Arg-Gly-Asp(OC_2H_5)-Cys-OH

Cyclo-S-acetyl-(D-Leu)-Arg-Gly-Asp(OC_2H_5)-Cys-OC_2H_5
Cyclo-S-acetyl-(D-Ile)-Arg-Gly-Asp-Cys-OC_2H_5
Cyclo-S-acetyl-(D-Ile)-Arg-Gly-Asp(OC_2H_5)-Cys-OH
Cyclo-S-acetyl-(D-Ile)-Arg-Gly-Asp(OC_2H_5)-Cys-OC_2H_5
Cyclo-S-acetyl-(D-Phe)-Arg-Gly-Asp-Cys-OC_2H_5
Cyclo-S-acetyl-(D-Phe)-Arg-Gly-Asp(OC_2H_5)-Cys-OH
Cyclo-S-acetyl-(D-Phe)-Arg-Gly-Asp(OC_2H_5)-Cys-OC_2H_5
Cyclo-S-acetyl-(D-Thr)-Arg-Gly-Asp-Cys-OC_2H_5
Cyclo-S-acetyl-(D-Thr)-Arg-Gly-Asp(OC_2H_5)-Cys-OH
Cyclo-S-acetyl-(D-Thr)-Arg-Gly-Asp(OC_2H_5)-Cys-OC_2H_5
Cyclo-S-acetyl-(D-Pro)-Arg-Gly-Asp-Cys-OC_2H_5
Cyclo-S-acetyl-(D-Pro)-Arg-Gly-Asp(OC_2H_5)-Cys-OH
Cyclo-S-acetyl-(D-Pro)-Arg-Gly-Asp(OC_2H_5)-Cys-OC_2H_5

Example 15

Cyclo-S-acetyl-(D-Tyr)-Arg-Gly-Asp-Cys-NH_2



Synthesis of the title compound was accomplished using standard Boc-synthetic protocols on a 4-methylbenzylhydrylamine resin to obtain first a linear peptide as described in Example 1. Cleavage of the linear peptide from the resin with hydrogen fluoride followed by cyclization as described in Example 2 affords the title compound after HPLC purification. HPLC retention time: 18 minutes. Molecular weight. Calc.: 651.2. Obs. (FAB mass spectrum): 652.2 (M+1).

Using this procedure the following compounds are analogously obtained.

Cyclo-S-acetyl-(D-Ala)-Arg-Gly-Asp-Cys-NH_2

Cyclo-S-acetyl-(D-Val)-Arg-Gly-Asp-Cys-NH_2

Cyclo-S-acetyl-(D-Leu)-Arg-Gly-Asp-Cys-NH_2

Cyclo-S-acetyl-(D-Ile)-Arg-Gly-Asp-Cys-NH_2

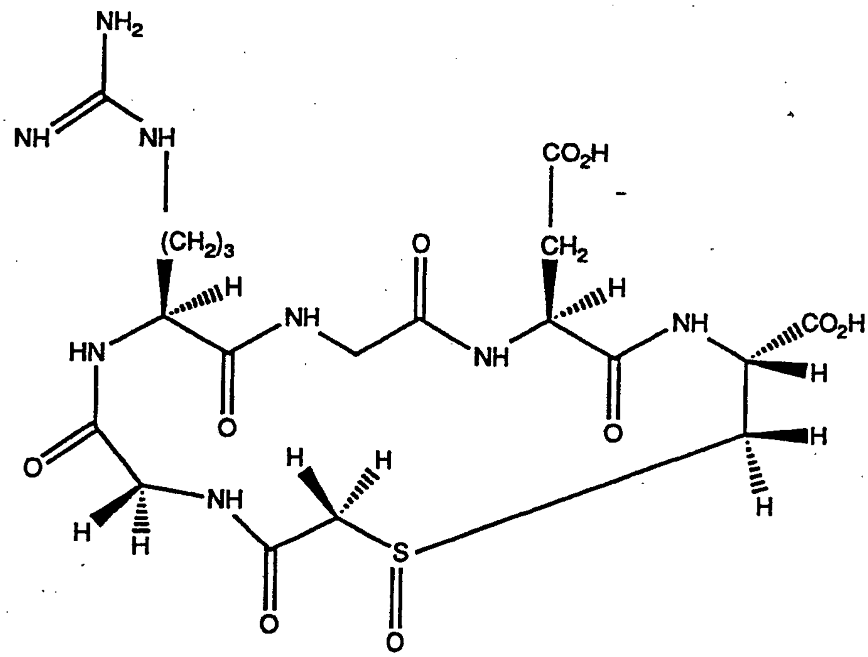
Cyclo-S-acetyl-(D-Phe)-Arg-Gly-Asp-Cys-NH_2

Cyclo-S-acetyl-(D-Pro)-Arg-Gly-Asp-Cys-NH_2

Cyclo-S-acetyl-Gly-Arg-Gly-Asp-Cys-NH_2

Example 16

Cyclo-S-acetyl-Gly-Arg-Gly-Asp-Cys-OH sulfoxide



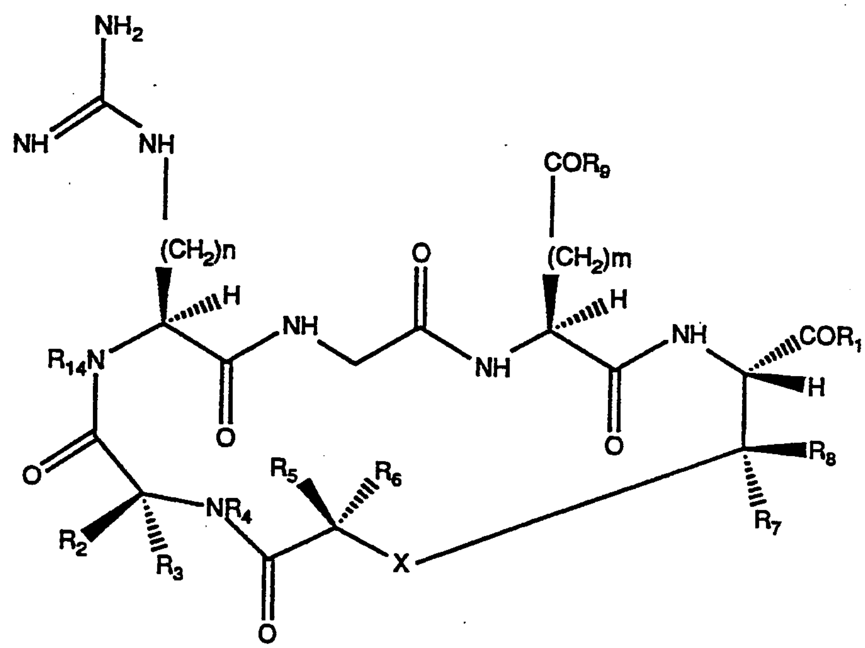
The purified product from Example 2 was dissolved in water at a concentration of 10 mg per mL. The pH of the solution was adjusted to 7. A 50% solution of hydrogen peroxide was added to make a final concentration of 3% hydrogen peroxide and the resulting reaction mixture was stirred overnight at room temperature. The solution was loaded directly onto an octadecylsilyl reverse phase chromatography column. The sulfoxide isomers formed in the reaction were eluted with a linear gradient of acetonitrile in 1% trifluoroacetic acid in water. Isomer 1 eluted at 3.5 minutes. Isomer 2 eluted at 4 minutes.

For both isomers: Molecular weight (calc.) 562.2. Found (FAB mass spectrum): isomer 1: 563.3; isomer 2: 563.3 (M+1).

Example 17

Preparation of cyclic thioether sulfoxides

Application of the procedure of Example 16 to thioether peptides prepared in Example 4 and shown in Table 1 and in to peptides prepared by the procedure in Example 15 affords the sulfoxide stereoisomers of Formula VIII listed in Table 3 where R_{14} is hydrogen, R_9 is OH, and X is SO.



VIII

Table 3

Sulfoxides of Formula VIII

R1	R2	R3	R4	R5, R6	R7	R8	MW[1] (calc)	MW[2] (obs)	RT[3]
OH	H	1-hydroxy-1-ethyl	H	H, H	H	H	606.2	607.1	5
OH	H	1-hydroxy-ethyl	H	H, H	H	H	606.2	607.1	7
OH	H	4-hydroxy-benzyl	H	H, H	CH_3	CH_3	680.2	681.1	15
OH	H	4-hydroxy-benzyl	H	H, H	CH_3	CH_3	680.2	681.1	20
OH	H	(4)	(4)	H, H	H	H	602.2	603.2	9.5
OH	H	(4)	(4)	H, H	H	H	602.2	603.2	10.5
OH	H	2-methyl-1-propyl	H	H, H	H	H	618.3	619.2	15
OH	H	2-methyl-1-propyl	H	H, H	H	H	618.3	619.2	19
OH	H	4-amino-1-butyl	H	H, H	H	H	633.2	634.0	5
OH	H	4-amino-1-butyl	H	H, H	H	H	633.2	634.0	8
OH	H	2-propyl	H	H, H	H	H	604.2	605.1	12
OH	H	2-propyl	H	H, H	H	H	604.2	605.1	14

OH	H	H	H	H, phenyl	H	H	638.2	639.0	16
OH	H	H	H	H, phenyl	H	H	638.2	639.0	18
NH_2	H	4-hydroxy-benzyl	H	H, H	H	H	667.2	668.3	12
NH_2	H	4-hydroxy-benzyl	H	H, H	H	H	667.2	668.3	15
OH	H	4-hydroxy-benzyl	H	H, H	H	H	668.2	669.2	12
OH	H	4-hydroxy-benzyl	H	H, H	H	H	668.2	669.2	16
OH	H	benzyl	H	H, H	H	H	652.2	653.3	17
OH	H	benzyl	H	H, H	H	H	652.2	653.3	20
OH	H	hydroxy-methyl	H	H, H	H	H	592.2	593.1	3
OH	H	hydroxy-methyl	H	H, H	H	H	592.2	593.1	5
OH	benzyl	H	H	H, H	H	H	652.2	653.3	20
OH	benzyl	H	H	H, H	H	H	652.2	653.3	23

Notes

(1) Calculated molecular weight of the peptide.

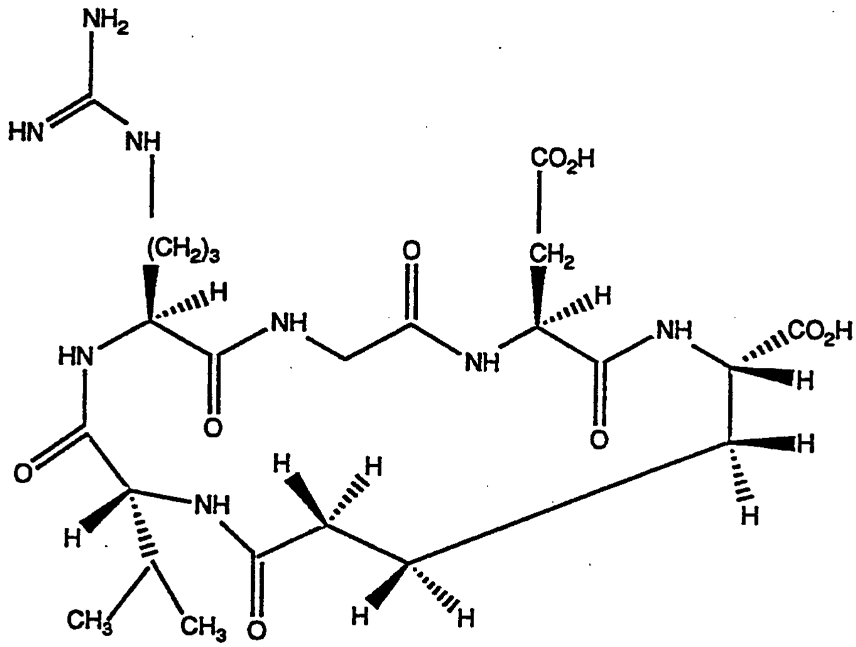
(2) Observed molecular weight for M+1, where M is the molecular ion, based on FAB mass spectrometry.

(3) Observed chromatographic retention time of the peptide in minutes determined using the conditions given in Example 2.

(4) $R_3 + R_4 = CH_2CH_2CH_2$.

Example 18

Synthesis of the compound of Formula IX



IX

α-FMOC-aminoadipic acid δ-allylester was coupled to Wang resin with diisopropylcarbodiimide and a catalytic amount of 4-dimethylaminopyridine in dichloromethane solvent. The FMOC-group was then removed with 20% piperidine in N,N-dimethylacetamide. Standard FMOC synthesis chemistry was then used to add the Asp, Gly, Arg, and D-Val residues. After the coupling of the D-Val residue, treatment of the resin-bound peptide with 0.3 equivalents of tetrakis(triphenylphosphine)palladium(0) in N,N-dimethylacetamide containing 20% piperidine resulted in removal of the allyl and FMOC groups. The resin-bound peptide was then washed 5 times with 5% N-methylmorpholine in N,N-dimethylacetamide. The peptide was then cyclized with 2 equivalents of BOP reagent. Cleavage of the peptide from the resin was accomplished by treatment with trifluoroacetic acid : triethylsilane (98:2). The peptide was purified by HPLC as described in Example 2. HPLC retention time: 14 minutes. Molecular weight: calc.: 556.3. obs.(FAB mass spectrum): 557.3.

Example 19

Synthesis of compounds of Formula VIII derived from glutamic or 2-aminoadipic acid

The compounds of Formula VIII in which R_1 and R_9 are OH, R_5, R_6, R_7, R_8, and R_{14} are hydrogen, the group X is $(CH_2)_k$ where k is 0 or 1, m is 1, and n is 3 are prepared using the procedure of Example 18. In Table 4 k = 0 means glutamic acid and k = 1 means aminoadipic acid.

Table 4

Cyclic Peptides Containing glutamic or 2-aminoadipic acid

R2	R3	R4	MW[1] (calc)	MW[2] (obs)	RT[3]	k
H	1-hydroxy-ethyl	H	558.7	559.3	10	0
H	2-propyl	H	556.3	557.0	14	0
H	2-methyl-1-propyl	H	570.3	571.0	22	0
H	3-indolyl-methyl	H	643.3	644.3	36	0
H	4-amino-1-butyl	H	585.3	586.3	7	0
H	benzyl	H	604.3	605.2	29	0
H	2-methylthio-1-ethyl	H	588.2	589.2	16	0
H	hydroxy-methyl	H	544.2	545.2	7	0
H	4-hydroxy-benzyl	H	620.2	621.0	18	0
H	2-carbox-amidoethyl	H	585.2	586.2	8	0
H	(4)	(4)	554.2	554.8	13	0
H	carboxamido-methyl	H	571.2	572.2	6	0
H	methyl	H	528.2	529.0	10	0
4-hydroxy-benzyl	H	H	620.2	621.0	18	0
H	carboxy-methyl	H	572.2	573.2	9	0
(5)	(5)	H	554.2	555.1	10	0
H	2-propyl	H	570.2	571.1	15	1
H	4-hydroxy-benzyl	H	634.2	635.1	16	1

Notes

(1) Calculated molecular weight of the peptide.

(2) Observed molecular weight for M+1, where M is the molecular ion, based on FAB mass spectrometry.

(3) Observed chromatographic retention time of the peptide in minutes determined using the conditions given in Example 2.

(4) $R_3 + R_4 = CH_2CH_2CH_2$

(5) $R_2 + R_4 = CH_2CH_2CH_2$.

Example 20

L-2-FMOC-Amino-3-(N-t-butoxycarbonyl-N-allyloxycarbonylmethyl)aminopropionic acid

Slow distillation of a solution of N-CBZ-D-serine methyl ester, dimethoxypropane (5 eq) and a catalytic amount of pyridinium p-toluenesulfonate (0.014 eq) in benzene resulted in the clean formation of the methyl 3-Cbz-2,2-dimethyloxazolidine-4- carboxylate. Reduction of the methyl ester with $LiBH_4$ (3 eq) in 2:1 ethanol-THF gave 3- Cbz-2,2-dimethyloxazolidine-4- methanol. Swern oxidation with $(COCl)_2$ (2 eq), DMSO (4 eq) and Et_3N (5 eq) in CH_2Cl_2 gave the 3- Cbz-2,2-dimethyloxazolidine-4- carboxyaldehyde which was reductively aminated with glycine benzyl ester hydrochloride (5 eq) and $NaBH_3CN$ (1 eq) in methanol to give 4- (N-benzyloxycarbonylmethyl)-aminomethyl-3- Cbz-2,2-dimethyloxazolidine. The resulting amine was protected as the Boc derivative using Boc_2O (1.25 eq) and $NaHCO_3$ (1.4 eq) in 2:1 THF-H_2O. Hydrogenation of a methanolic solution of 4- (N-t-butyloxycarbonyl-N-benzyloxycarbonylmethyl)-aminomethyl-3- Cbz-2,2-dimethyloxazolidine, obtained in the previous step, at 50 psi in the presence of 10% Pd-C, the CBZ and benzyl ester groups were cleaved and the oxazolidine ring hydrolyzed, giving L-2-amino-3-(N-t-butoxycarbonyl-N-carboxymethyl)amino-1-propanol. The FMOC group was appended to the 2-amino function using N-(9-fluorenylmethoxycarbonyloxy)succinimide (1.15 eq) and $NaHCO_3$ (2.5 eq) in 1:1 dioxane-H_2O. Allyl esterification of the ω-carboxyl group using allyl bromide (7 eq) and $NaHCO_3$ (2.5 eq) in DMF followed by Jones oxidation (3 eq) of the primary alcohol gave the desired title compound.

Example 21

N-FMOC-O-allyloxycarbonylmethyl-L-serine

O-allylation of N-CBZ-L-serine in DMF solution was achieved by sequential treatment with NaH (2.2 eq) and allyl bromide (1.1 eq). The carboxylic acid was converted to the *tert*-butyl ester using isobutylene and H_2SO_4 in methylene chloride to afford O-allyl-N-Cbz-L-serine t-butyl ester. Cleavage of the terminal olefin of the allyl group by ozonolysis in methanol with a dimethylsulfide workup gave the aldehyde which, without isolation, was reduced to O-(2-hydroxy-1-ethyl)- N-Cbz-L-serine t-butyl ester with $NaBH_4$ (1 eq). The FMOC group was introduced by first reductively cleaving the CBZ group at 50 psi H_2 in methanol over 10% Pd-C catalyst, then reprotecting the resulting free amine with fluorenylmethylchloroformate (1.15 eq) and $NaHCO_3$ (1.8 eq) in 2:1 THF-H_2O. Jones oxidation (3 eq) of the alcohol on the hydroxyethyl group gave FMOC-O-carboxymethyl-L-serine-t-butyl ester, which was treated with allyl bromide (3 eq) and $NaHCO_3$ (1.5 eq) in DMF to give FMOC-O-allyloxycarbonylmethyl-L-serine-t-butyl ester. Finally, the t-butyl ester was removed with trifluoroacetic acid giving the desired N-FMOC-O-allyloxycarbonylmethyl-L-serine.

Example 22

Synthesis of cyclic peptides of Formula VIII derived from O-carboxymethyl-L-serine and 3-(N-carboxymethyl)-2,3-diaminopropionic acid

The protected amino acids from Examples 20 and 21 were coupled to Wang resin using diisopropylcarbodiimide and catalytic 4-dimethylaminopyridine. Standard FMOC solid phase synthesis protocol

was used to couple the Asp, Gly, Arg and selected D-amino acid residues. Thus, coupling steps were carried out using BOP and N-methyl morpholine in N,N-dimethylacetamide. Deprotections were performed with 20% piperidine in N,N-dimethylacetamide. Cleavage of the allyl ester groups was effected with $(Ph_3P)_4Pd$ in 20% piperidine/N,N-dimethylacetamide. The peptide was cyclized while still attached to the resin using diisopropylcarbodiimide and a catalytic amount of N-hydroxybenztriazole in dichloromethane to effect cyclization. The cyclized peptides were cleaved from the resin with trifluoroacetic acid and purified on reverse phase HPLC. The peptides prepared by this route are derivatives of Formula VIII wherein R_1 and R_9 are OH, R_2, R_4, R_5, R_6, R_7, R_8, and R_{14} are hydrogen, n is 3 and m is 1 and the remaining substituents are as shown.

R_3	X	MW(calc)[1]	MW(obs)[2]	RT[3]
2-propyl	NH	571.2	572.3	8
4-hydroxybenzyl	NH	635.2	636.3	25
4-hydroxybenzyl	O	636.2	637.2	18

Notes

(1) Calculated molecular weight of the peptide.

(2) Observed molecular weight for M+1, where M is the molecular ion, based on FAB mass spectrometry.

(3) Observed chromatographic retention time of the peptide in minutes determined using the conditions given in Example 2.

Example 23

Inhibition of fibrinogen binding to GP IIbIIIa

Microtiter plates are coated with fibrinogen (10 μg/ml) and then blocked with TACTS buffer containing 0.5% BSA. (TACTS buffer contains 20mM Tris.HCl, pH 7.5, 0.02% sodium azide, 2 mM calcium chloride, 0.05% Tween 20, 150 mM sodium chloride.) The plate is washed with phosphate buffered saline containing 0.01% Tween 20 and a dilution of the sample to be determined added, followed by addition of solubilized IIbIIIa receptor (40 μg/ml) in TACTS, 0.5% BSA. After incubation, the plate is washed and murine monoclonal anti-platelet antibody AP3 (1 μg/ml) added. After another wash goat and anti-mouse IgG conjugated to horseradish peroxidase is added. A final wash is performed and developing reagent buffer (10 mg o-phenylenediamine dihydrochloride, 0.0212% hydrogen peroxide, 0.22 mM citrate, 50 mM phosphate, pH 5.0) is added and then incubated until color developed. The reaction is stopped with 1N sulfuric acid and the absorbance at 492 nm is recorded and the IC_{50} values determined. The results are recorded in Table 5.

Table 5

Inhibition of Fibrinogen Binding to GPIIbIIIa by Compounds of Formula VIII

R_1	R_2	R_3	R_4	R_5, R_6	R_7	R_8	R_9	X	FBI *
OH	H	(2)	(2)	H, H	H	H	OH	S	0.95

OH	H	1-hydroxy-1-ethyl	H	H, H	H	H	OH	S	1.3
OH	H	H	H	H, 1-naphthyl	H	H	OH	S	1.4 (5)
OH	H	H	H	H, H	H	H	OH	SO	1.4 (7)
OH	H	4-hydroxy-benzyl	H	H, H	H	H	OH	SO	1.5 (7)
OH	H	1-hydroxy-1-ethyl	H	H, phenyl	H	H	OH	S	1.7 (5)
OH	H	1-hydroxy-1-ethyl	H	H, H	H	H	OH	SO	1.8 (7)
OH	H	H	H	H, 4-biphenyl	H	H	OH	S	1.9 (5)
OH	H	4-hydroxy-benzyl	H	H, H	CH_3	CH_3	OH	SO	1.9 (7)
OH	H	4-hydroxy-benzyl	H	H, H	H	H	OH	S	2
OH	H	H	H	H, phenyl	H	H	OH	S	2 (5)
OH	H	H	H	H, H	CH_3	CH_3	OH	S	2
OH	H	2-propyl	H	H, H	H	H	OH	S	2
OH	H	(2)	(2)	H, H	H	H	OH	SO	2.1 (7)
OH	H	2-propyl	H	H, H	H	H	OH	CH_2	2.2
OH	H	2-methyl-1-propyl	H	H, H	H	H	OH	SO	2.3 (7)
OH	H	methyl	H	H, H	H	H	OH	S	2.5
NH_2	H	4-hydroxy-benzyl	H	H, H	H	H	OH	S	2.5
OH	H	4-amino-1-butyl	H	H, H	H	H	OH	SO	2.6 (7)
OH	H	carboxamido methyl	H	H, H	H	H	OH	S	2.7
OH	H	H	H	H, 2-tri-fluoromethyl-phenyl	H	H	OH	S	2.7 (5)
OH	H	2-propyl	H	H, H	H	H	OH	SO	2.9 (7)
OH	H	4-imidazolyl-methyl	H	H, H	H	H	OH	S	3.0

OH	H	2-methyl-1-propyl	H	H, H	H	H	OH	S	3.0
OH	H	4-hydroxy-benzyl	H	H, phenyl	H	H	OH	S	3.0
OH	H	H	H	H, 4-biphenyl	H	H	OH	S	3.5 (6)
OH	H	4-hydroxy-benzyl	H	H, H	H	H	OH	CH_2	3.5
OH	H	H	H	H, 2-naphthyl	H	H	OH	S	3.8 (5)
OH	H	2-methyl-thioethyl	H	H, H	H	H	OH	S	4.0
OH	H	hydroxy-methyl	H	H, H	H	H	OH	S	4.0
OH	H	3-indolyl-methyl	H	H, H	H	H	OH	S	4.0
OH	H	carboxamido methyl	H	H, H	H	H	OH	S	4.0
OH	H	H	H	H, 4-tri-fluoromethyl phenyl	H	H	OH	S	4.0 (5)
OH	H	H	H	H, phenyl	H	H	OH	SO	4.0 (5)
OH	H	H	H	H, H	H	H	OH	S	4.2
OH	H	2-propyl	H	H, H	H	H	OH	NH	4.3
OH	H	2-propyl	H	H, H	H	H	OH	SO	4.5 (8)
NH_2	H	4-hydroxy-benzyl	H	H, H	H	H	OH	SO	4.5 (7)
OH	H	4-hydroxy-benzyl	H	H, H	H	H	OH	SO	4.8 (8)
OH	H	H	H	H, 1-naphthyl	H	H	OH	S	5.3 (6)
OH	H	H	H	H, 1-propyl	H	H	OH	S	5.4 (5)
OH	H	3-amino-1-propyl	H	H, H	H	H	OH	S	5.6
OH	benzyl	H	H	H, H	H	H	OH	SO	5.6 (7)
OH	H	H	H	H, 3-tri-fluoromethyl phenyl	H	H	OH	S	5.7 (5)

OH	H	H	H	H, ethyl	H	H	OH	S	6.0 (5)
OH	H	2-butyl	H	H, H	H	H	OH	S	6.0
OH	H	benzyl	H	H, H	H	H	OH	SO	6.6 (7)
OH	4-imida-zolyl-methyl	H	H	H, H	H	H	OH	S	6.8
OH	benzyl	H	H	H, H	H	H	OH	S	7.0
OH	H	1-hydroxy-1-ethyl	H	H, phenyl	H	H	OH	S	7.0 (6)
OH	H	4-hydroxy-benzyl	H	H, H	CH_3	CH_3	OH	S	7.0
OH	H	(2)	(2)	H, H	H	H	OH	SO	7.0 (8)
OH	H	4-amino-1-butyl	H	H, H	H	H	OH	S	7.5
OH	H	4-hydroxy-benzyl	H	H, H	H	H	OH	NH	8.0
OH	H	hydroxy-methyl	H	H, H	H	H	OH	SO	8.0
OH	CH_3	H	H	H, H	H	H	OH	S	9.5
OH	H	2-propyl	H	H, H	H	H	OH	O	9.7
OH	H	1-hydroxy-1-ethyl	H	H, H	H	H	OH	SO	10 (8)
OH	(3)	H	(3)	H, H	H	H	OH	S	11.4
OH	H	H	H	H, 2-naphthyl	H	H	OH	S	11.5 (6)
OH	1-hydroxy-1-ethyl	H	H	H, H	H	H	OH	S	11.6
eth-oxy	H	4-hydroxy-benzyl	H	H, H	H	H	OH	S	12
OH	H	4-amino-1-butyl	H	H, H	H	H	OH	SO	14 (8)
OH	H	H	H	H, phenyl	H	H	OH	S	15 (6)
OH	H	2-carboxy-1-ethyl	H	H, H	H	H	OH	S	15
OH	H	4-hydroxy-benzyl	H	H, H	CH_3	CH_3	OH	SO	15 (8)

OH	1-butyl	H	H	H, H	H	H	OH	S	16
OH	H	benzyl	H	H, H	H	H	OH	SO	16 (8)
OH	2-propyl	H	H	H, H	H	H	OH	S	17
OH	2-butyl	H	H	H, H	H	H	OH	S	18
OH	H	H	H	H, H	H	H	OH	SO_2	18
OH	4-hydroxy-benzyl	H	H	H,H	H	H	OH	S	20
OH	H	H	H	H, ethyl	H	H	OH	S	20 (6)
OH	4-amino-1-butyl	H	H	H, H	H	H	OH	S	20
OH	H	4-hydroxy-benzyl	H	H, H	H	H	OH	O	20
OH	H	H	H	H, 1-propyl	H	H	OH	S	21 (6)
OH	hydroxy-methyl	H	H	H, H	H	H	OH	S	22
OH	H	H	H	H, 2-tri-fluoromethyl phenyl	H	H	OH	S	23 (6)
OH	H	carboxy-methyl	H	H, H	H	H	OH	S	25
OH	H	H	H	H, H	H	H	OH	SO	25 (8)
OH	2-methyl-thioethyl	H	H	H, H	H	H	OH	S	28
OH	3-indolyl-methyl	H	H	H, H	H	H	OH	S	29
OH	3-amino-1-propyl	H	H	H, H	H	H	OH	S	31
OH	benzyl	H	H	H, H	H	H	OH	SO	33 (8)
OH	carbox-amido methyl	H	H	H, H	H	H	OH	S	35
OH	benzyl	H	H	H, H	H	H	OH	S	35
OH	H	H	H	H, 3-tri-fluoromethyl phenyl	H	H	OH	S	35 (6)

OH	isobutyl	H	H	H, H	H	H	OH	S	38
OH	H	H	H	H, CH_3	H	H	OH	S	39
NH_2	H	4-hydroxy-benzyl	H	H, H	H	H	OH	SO	39 (8)
OH	H	H	H	H, H	H	H	OH	O	42
OH	H	H	H	H, phenyl	H	H	OH	SO	43 (6)
OH	H	hydroxy-methyl	H	H, H	H	H	OH	SO	50 (8)
OH	H	2-methyl-1-propyl	H	H, H	H	H	OH	SO	50 (8)
OH	2-carbox-amido ethyl	H	H	H, H	H	H	OH	S	110
eth-oxy	H	4-hydroxy-benzyl	H	H, H	H	H	ethoxy	S	158
OH	H	H	H	H, 4-tri-fluoromethyl phenyl	H	H	OH	S	180 (6)
OH	2-carboxy-ethyl	H	H	H, H	H	H	OH	S	260
OH	carboxy-methyl	H	H	H, H	H	H	OH	S	500
OH	H	4-hydroxy-benzyl	H	H, H	H	H	ethoxy	S	500
OH	H	1-hydroxy-ethyl	H	H, H	H	H	OH	(4)	1.05
OH	H	2-propyl	H	H, H	H	H	OH	(4)	2.9
OH	H	isobutyl	H	H, H	H	H	OH	(4)	3.0
OH	H	3-indolyl-methyl	H	H, H	H	H	OH	(4)	4.0
OH	H	4-amino-1-butyl	H	H, H	H	H	OH	(4)	5.0
OH	H	benzyl	H	H, H	H	H	OH	(4)	6.0
OH	H	2-methylthio-1-ethyl	H	H, H	H	H	OH	(4)	8.0

OH	H	hydroxy-methyl	H	H, H	H	H	OH	(4)	10
OH	H	4-hydroxy-benzyl	H	H, H	H	H	OH	(4)	11.2
OH	H	2-carbox-amidoethyl	H	H, H	H	H	OH	(4)	12
OH	H	(2)	(2)	H, H	H	H	OH	(4)	12
OH	H	carboxamido-methyl	H	H, H	H	H	OH	(4)	25
OH	H	methyl	H	H, H	H	H	OH	(4)	41.5
OH	4-hydroxy-benzyl	H	H	H, H	H	H	OH	(4)	60
OH	H	carboxy-methyl	H	H, H	H	H	OH	(4)	67
OH	(3)	(3)	H	H, H	H	H	OH	(4)	360

* Fibrinogen Binding Inhibition[1] IC_{50} (nM)

Notes

(1) Fibrinogen binding inhibition assay data is from Example 23. The IC_{50} values are in nanomolar units.

(2) $R_3 + R_4 = CH_2CH_2CH_2$

(3) $R_2 + R_4 = CH_2CH_2CH_2$

(4) $X = (CH_2)_k$ and k is 0.

(5) IC_{50} for the more active isomer at R_5/R_6

(6) IC_{50} for the less active isomer at R_5/R_6.

(7) IC_{50} for the more active sulfoxide isomer

(8) IC_{50} for the less active sulfoxide isomer.

Example 24

Inhibition of platelet aggregation

Fifty milliliters of whole human blood (9 parts) is drawn on 3.6% sodium citrate (1 part) from a donor who has not taken aspirin or related medications for at least two weeks. The blood is centrifuged at 160 x g for 10 min at 22° C and then allowed to stand for 5 min after which the PRP is decanted. Platelet poor plasma (PPP) is isolated from the remaining blood after centrifugation at 2000 x g for 25 min. The platelet count of the PRP was diluted to ca. 300000 per microliter with PPP.

A 225 µL aliquot of PRP plus 25 µL of either a dilution of the test sample or a control (PBS) is incubated for 5 min in a Chrono-log Whole Blood Aggregometer at 25° C. Adenosine diphosphate (ADP, 8 µM) is added and the platelet aggregation recorded. The results of this test are recorded in Table 6. Where the possibility of stereoisomers exists, the values in Table 6 are for the most active stereoisomers.

Table 6

Inhibition of Platelet Aggregation by Compounds of Formula VIII

R_1	R_2	R_3	R_4	R_5, R_6	R_7	R_8	R_9	X	PAI *
OH	H	2-propyl	H	H, H	H	H	OH	CH_2	0.09
OH	H	H	H	H, 1-naphthyl	H	H	OH	S	0.11
OH	H	4-hydroxy-benzyl	H	H, H	H	H	OH	SO	0.13
OH	H	4-hydroxy-benzyl	H	H, H	H	H	OH	CH_2	0.15
OH	H	4-hydroxy-benzyl	H	H, H	CH_3	CH_3	OH	SO	0.17
OH	H	2-propyl	H	H, H	H	H	OH	SO	0.19
OH	H	4-hydroxy-benzyl	H	H, H	CH_3	CH_3	OH	S	0.2
NH_2	H	4-hydroxy-benzyl	H	H, H	H	H	OH	SO	0.20
OH	H	1-hydroxy-1-ethyl	H	H, phenyl	H	H	OH	S	0.30
OH	H	benzyl	H	H, H	H	H	OH	SO	0.36
OH	H	2-methyl-1-propyl	H	H, H	H	H	OH	(3)	0.40
OH	H	1-hydroxy-1-ethyl	H	H, H	H	H	OH	SO	0.44
OH	H	2-propyl	H	H, H	H	H	OH	S	0.44
OH	H	4-amino-1-butyl	H	H, H	H	H	OH	SO	0.46
OH	H	2-propyl	H	H, H	H	H	OH	(3)	0.70
NH_2	H	4-hydroxy-benzyl	H	H, H	H	H	OH	S	0.71
OH	H	H	H	H, 2-naphthyl	H	H	OH	S	0.71
OH	H	2-propyl	H	H, H	H	H	OH	NH	0.73
OH	H	1-hydroxy-1-ethyl	H	H, H	H	H	OH	S	0.8
OH	H	4-hydroxy-benzyl	H	H, H	H	H	OH	S	0.8
OH	H	H	H	H, phenyl	H	H	OH	S	0.87
OH	H	4-hydroxy-benzyl	H	H, H	H	H	OH	(3)	1.0
OH	H	2-methyl-1-propyl	H	H, H	H	H	OH	S	1.04
OH	H	benzyl	H	H, H	H	H	OH	(3)	1.1
OH	H	4-hydroxy-benzyl	H	H, phenyl	H	H	OH	S	1.25
OH	H	3-indolyl-methyl	H	H, H	H	H	OH	(3)	1.3
OH	H	benzyl	H	H, H	H	H	OH	S	1.35
OH	H	4-hydroxy-benzyl	H	H, H	H	H	OH	NH	1.46
OH	H	1-hydroxy-ethyl	H	H, H	H	H	OH	(3)	1.5
OH	H	H	H	H, H	CH_3	CH_3	OH	S	1.7
OH	H	H	H	H, H	H	H	OH	SO	1.9
OH	H	(2)	(2)	H, H	H	H	OH	S	2.0

OH	H	3-indolyl-methyl	H	H, H	H	H	OH	S	2.3
OH	H	hydroxy-methyl	H	H, H	H	H	OH	S	2.45
OH	H	4-imidazolyl-methyl	H	H, H	H	H	OH	S	2.77
OH	H	methyl	H	H, H	H	H	OH	S	3.0
OH	H	2-methyl-thioethyl	H	H, H	H	H	OH	S	5.4
OH	H	4-hydroxy-benzyl	H	H, H	CH_3	CH_3	OH	SO	5.4
OH	H	H	H	H, H	H	H	OH	S	6.6
OH	H	H	H	H, 4-biphenyl	H	H	OH	S	7.56
OH	H	(2)	(2)	H, H	H	H	OH	(3)	10.2
OH	H	H	H	H, H	H	H	OH	SO_2	39.5
OH	H	H	H	H, H	H	H	OH	SO	<20

* Platelet Aggregation Inhibition[1] IC_{50} (μM)

Notes

(1) Platelet aggregation inhibition assay data is from Example 24. The IC_{50} values are in micromolar units.

(2) $R_3 + R_4 = CH_2CH_2CH_2$

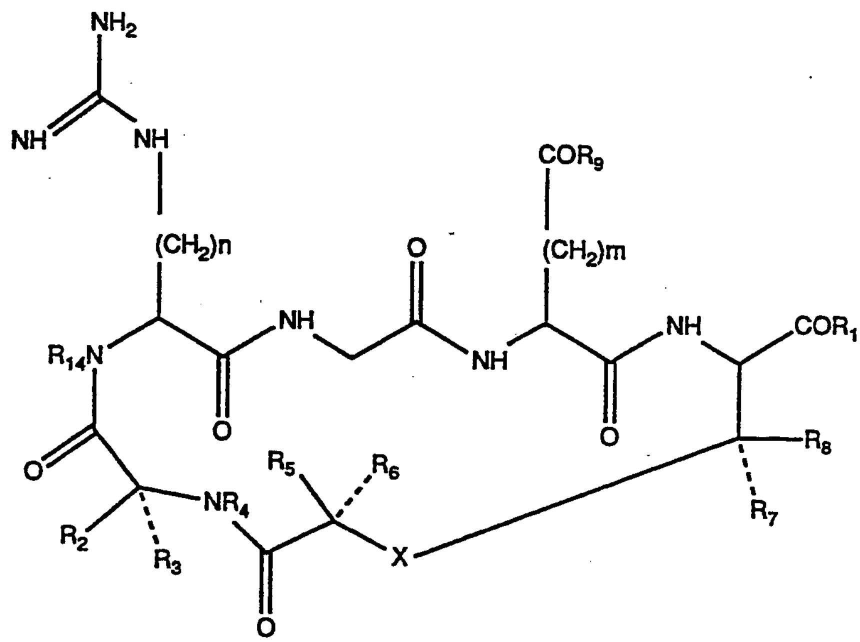
(3) $X = (CH_2)_k$ and k is 0.

In view of the efficacy of these cyclic polypeptides as inhibitors of fibrinogen binding to GP IIbIIIa, and the feasibility as demonstrated herein of producing these cyclic polypeptides, the present invention may have application in the treatment of a large group of disorders associated with, or characterized by, a hyperthrombotic state. Representative of such disorders are genetic or acquired deficiencies of factors which normally prevent a hyperthrombotic state; medical procedures such as angioplasty and thrombolytic therapy; mechanical obstructions to blood flow, such as tumor masses, prosthetic synthetic cardiac valves, and extracorporeal perfusion devices; atherosclerosis; and coronary artery disease.

The present invention has of necessity been discussed herein by reference to certain specific methods and materials. It is to be understood that the discussion of these specific methods and materials in no way constitutes any limitation on the scope of the present invention, which extends to any and all alternative materials and methods suitable for accomplishing the objectives of the present invention.

What is claimed is:

1. A compound of the formula:



wherein

R_1 and R_9 are the same or different and are selected from

hydroxy,

C_1-C_8 alkoxy,

C_3-C_{12} alkenoxy,

C_6-C_{12} aryloxy,

di-C_1-C_8 alkylamino-C_1-C_8-alkoxy,

acylamino-C_1-C_8-alkoxy selected from the group acetylaminoethoxy, nicotinoylaminoethoxy, and succinamidoethoxy,

pivaloyloxyethoxy,

C_6-C_{12} aryl-C_1-C_8-alkoxy where the aryl group is unsubstituted or substituted with one or more of the groups nitro, halo (F, Cl, Br, I), C_1-C_4-alkoxy, and amino,

hydroxy-C_2-C_8-alkoxy,

dihydroxy-C_3-C_8-alkoxy, and

$NR_{10}R_{11}$ wherein R_{10} and R_{11} are the same or different and are hydrogen, C_1-C_8-alkyl, C_3-C_8-alkenyl, C_6-C_{12}-aryl where the aryl group is unsubstituted or substituted with one or more of the groups

nitro, halo (F, Cl, Br, I), C_1-C_4-alkoxy, and amino, C_6-C_{12}-aryl-C_1-C_8-alkyl where the aryl group is unsubstituted or substituted by one or more of the groups nitro, halo (F, Cl, Br, I), C_1-C_4-alkoxy, and amino;

R_2, R_3, R_5, R_6, R_7, R_8 are the same or different and are selected from

hydrogen,

C_6-C_{12} aryl where the aryl group is unsubstituted or substituted by one or more of the groups nitro, hydroxy, halo (F, Cl, Br, I), C_1-C_8 alkyl, halo-C_1-C_8 alkyl, C_1-C_8-alkoxy, amino, phenyloxy, phenyl, acetamido, benzamido, di-C_1-C_8 alkylamino, C_1-C_8 alkylamino, C_6-C_{12} aroyl, C_1-C_8 alkanoyl, and hydroxy-C_1-C_8 alkyl,

C_1-C_{12} alkyl either substituted or unsubstituted, branched or straight chain where the substituents are selected from halo (F, Cl, Br, I),

C_1-C_8 alkoxy,

C_6-C_{12} aryloxy where the aryl group is unsubstituted or substituted by one or more of the groups nitro, hydroxy, halo (F, Cl, Br, I), C_1-C_8 alkyl, C_1-C_8-alkoxy, amino, phenyloxy, acetamido, benzamido, di-C_1-C_8 alkylamino, C_1-C_8 alkylamino, C_6-C_{12} aroyl, and C_1-C_8 alkanoyl,

isothioureido,

C_3-C_7 cycloalkyl,

ureido,

amino,

C_1-C_8 alkylamino,

di-C_1-C_8 alkylamino,

hydroxy,

amino-C_2-C_8 alkylthio,

amino-C_2-C_8 alkoxy,

acetamido,

benzamido wherein the phenyl ring is unsubstituted or substituted by one or more of the groups nitro, hydroxy, halo (F, Cl, Br, I), C_1-C_8 alkyl, C_1-C_8-alkoxy, amino, phenyloxy, acetamido, benzamido, di-C_1-C_8 alkylamino, C_1-C_8 alkylamino, C_6-C_{12} aroyl, and C_1-C_8 alkanoyl,

C_6-C_{12} arylamino wherein the aryl group is unsubstituted or substituted by one or more of the groups nitro, hydroxy, halo, C_1-C_8 alkyl, C_1-C_8-alkoxy, amino, phenyloxy, acetamido, benzamido, di-C_1-C_8 alkylamino, C_1-C_8 alkylamino, C_6-C_{12} aroyl, and C_1-C_8 alkanoyl,

guanidino,

phthalimido,

mercapto,

C_1-C_8 alkylthio,

C_6-C_{12} arylthio,

carboxy,

carboxamide,

carbo-C_1-C_8 alkoxy,

C_6-C_{12} aryl wherein the aryl group is unsubstituted or substituted by one or more of the groups nitro, hydroxy, halo, C_1-C_8 alkyl, C_1-C_8-alkoxy, amino, phenyloxy, acetamido, benzamido, di-C_1-C_8 alkylamino, C_1-C_8 alkylamino, hydroxy-C_1-C_8 alkyl, C_6-C_{12} aroyl, and C_1-C_8 alkanoyl, and

aromatic heterocycle wherein the heterocyclic groups have 5-10 ring atoms and contain up to two O, N, or S heteroatoms;

R_2 and R_3, R_5 and R_6, or R_7 and R_8 may optionally and independently be joined together to form a carbocyclic or heterocyclic ring of from four to seven atoms where the heteroatoms are selected from O, S or NR_{12} where R_{12} is selected from

hydrogen, C_1-C_8-alkyl, C_3-C_8-alkenyl, C_6-C_{12}-aryl, C_6-C_{12}-aryl-C_1-C_8-alkyl, C_1-C_8 alkanoyl, and C_6-C_{12} aroyl,

R_4 is selected from

hydrogen,

C_1-C_8 alkyl,

C_3-C_{10} cycloalkyl,

C_6-C_{12} aryl, and

C_6-C_{12} aryl-C_1-C_8-alkyl;

R_2 or R_3 may be optionally joined with R_4 to form a piperidine, pyrrolidine or thiazolidine ring;

R_{14} is selected from

hydrogen, C_1-C_8-alkyl, C_3-C_8-alkenyl, C_6-C_{12}-aryl, and C_6-C_{12} aryl-C_1-C_8-alkyl;

X is selected from

an O or S atom,

an S atom bearing one or two O atoms,

NR_{13} wherein R_{13} is hydrogen, C_1-C_8-alkyl, C_3-C_8-alkenyl, C_6-C_{12}-aryl, C_6-C_{12}-aryl-C_1-C_8-alkyl, C_1-C_8 alkanoyl, and C_6-C_{12} aroyl, and

C_6-C_{12} aryl,

C_1-C_8 alkanoyl,

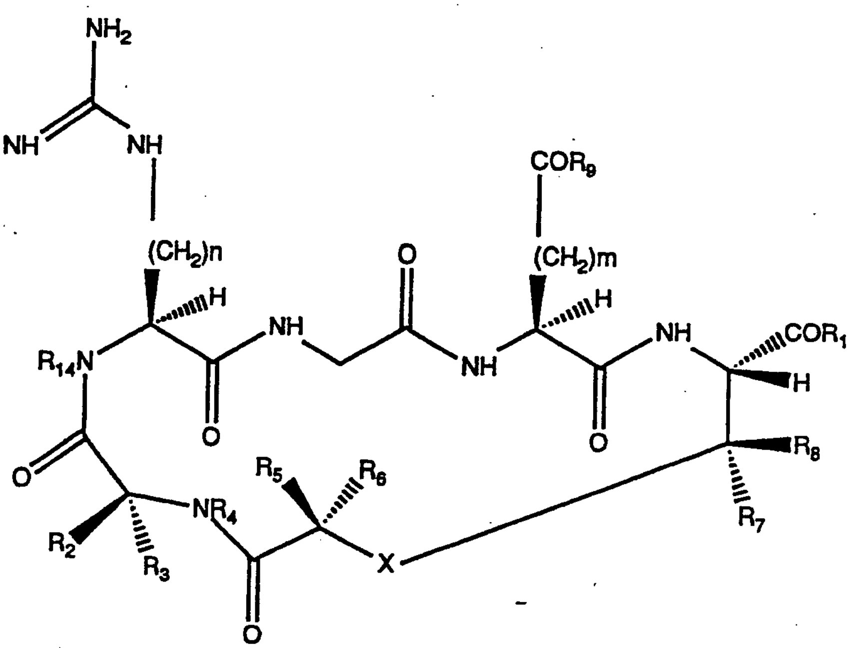
$(CH_2)_k$ where k is an integer from 0 to 5;

n is an integer from 1 to 6;

m is an integer from 0 to 4; and

pharmaceutically acceptable salts thereof.

2. A compound of the formula



wherein

R_1 and R_9 are the same or different and are hydroxy, NH_2, C_1-C_4 alkoxy or benzyloxy;

R_2 is hydrogen

R_3 is selected from

hydrogen,

C_1-C_6 alkyl branched or unbranched, unsubstituted or substituted with substituents selected from amino, hydroxy, mercapto, methylthio, carboxy, carboxamide, guanidino, phenyl, 4-hydroxyphenyl, 4-methoxyphenyl, 3-indolyl, and 4-imidazolyl,

phenyl either unsubstituted or substituted with one to three substituents that may be independently nitro, hydroxy, halo (F, Cl, Br, I), C_1-C_4 alkyl, C_1-C_4-alkoxy, amino, phenyloxy, phenyl, acetamido, benzamido, di-C_1-C_4 alkylamino, C_1-C_4 alkylamino, halo-C_1-C_4 alkyl, C_6-C_{12} aroyl, and C_1-C_4 alkanoyl,

1-naphthyl,

2-naphthyl,

2-thienyl,

2-pyridyl,

3-pyridyl, and

4-pyridyl;

R_5 and R_6 are independently selected from

hydrogen,

C_1-C_6 alkyl either branched or unbranched, unsubstituted or substituted with substituents selected from amino, hydroxy, mercapto, carboxy, carboxamide, guanidino, phenyl or 4-hydroxyphenyl, 4-methoxyphenyl, 3-indolyl, and 4-imidazolyl,

phenyl either unsubstituted or substituted with one to three substituents that may be independently selected from nitro, hydroxy, halo (F, Cl, Br, I), C_1-C_4 alkyl, C_1-C_4-alkoxy, amino, phenyloxy, phenyl, acetamido, benzamido, di-C_1-C_4 alkylamino, C_1-C_4 alkylamino, halo-C_1-C_4 alkyl, C_6-C_{12} aroyl, and C_1-C_4 alkanoyl,

1-naphthyl,

2-naphthyl,

2-thienyl,

2-pyridyl,

3-pyridyl, and

4-pyridyl;

R_7 or R_8 are the same or different and are selected from

hydrogen,

C_1-C_4 alkyl,

phenyl either unsubstituted or substituted with from one to three substituents independently selected from hydroxy, halo (F, Cl, Br, I), C_1-C_4 alkyl, and C_1-C_4-alkoxy;

R_4 is hydrogen or may be joined with R_3 to form a heterocyclic ring selected from piperidine, pyrrolidine or thiazolidine;

R_{14} is hydrogen or methyl;

X is selected from

an O or S atom,

an S atom bearing one or two O atoms,

NR_{13} where R_{13} is selected from hydrogen, C_1-C_4 alkyl, benzyl, phenyl, C_1-C_4 alkanoyl, benzoyl and

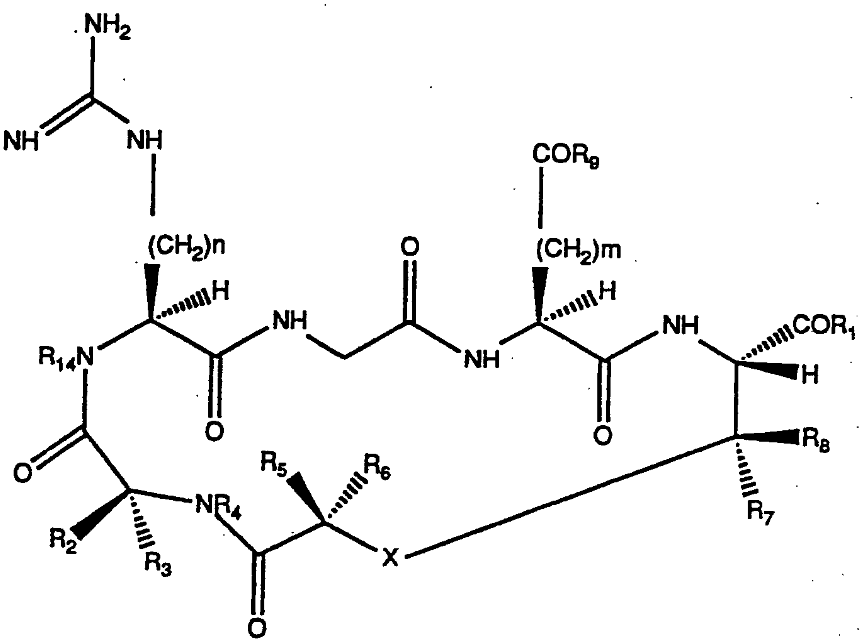
$(CH_2)_k$, where k is 0 to 5;

n is 3 or 4;

m is 1; and

pharmaceutically acceptable salts thereof.

3. A compound of the formula



wherein

R_1 and R_9 are the same or different and are hydroxy, NH_2, C_1-C_4 alkoxy or benzyloxy;

R_3 is hydrogen

R_2 is selected from

- hydrogen,
- C_1-C_6 alkyl branched or unbranched, unsubstituted or substituted with substituents selected from amino, hydroxy, mercapto, methylthio, carboxy, carboxamide, guanidino, phenyl, 4-hydroxyphenyl, 4-methoxyphenyl, 3-indolyl, and 4-imidazolyl,
- phenyl either unsubstituted or substituted with one to three substituents that may be independently nitro, hydroxy, halo (F, Cl, Br, I), C_1-C_4 alkyl, C_1-C_4-alkoxy, amino, phenyloxy, phenyl, acetamido, benzamido, di-C_1-C_4 alkylamino, C_1-C_4 alkylamino, halo-C_1-C_4 alkyl, C_6-C_{12} aroyl, and C_1-C_4 alkanoyl,
- 1-naphthyl,
- 2-naphthyl,
- 2-thienyl,
- 2-pyridyl,
- 3-pyridyl, and
- 4-pyridyl;

R_5 and R_6 are independently selected from

- hydrogen,

C_1-C_6 alkyl either branched or unbranched, unsubstituted or substituted with substituents selected from amino, hydroxy, mercapto, carboxy, carboxamide, guanidino, phenyl or 4-hydroxyphenyl, 4-methoxyphenyl, 3-indolyl, and 4-imidazolyl,

phenyl either unsubstituted or substituted with one to three substituents that may be independently selected from nitro, hydroxy, halo (F, Cl, Br, I), C_1-C_4 alkyl, C_1-C_4-alkoxy, amino, phenyloxy, phenyl, acetamido, benzamido, di-C_1-C_4 alkylamino, C_1-C_4 alkylamino, halo-C_1-C_4 alkyl, C_6-C_{12} aroyl, and C_1-C_4 alkanoyl,

1-naphthyl,

2-naphthyl,

2-thienyl,

2-pyridyl,

3-pyridyl, and

4-pyridyl;

R_7 or R_8 are the same or different and are selected from

hydrogen,

C_1-C_4 alkyl,

phenyl either unsubstituted or substituted with from one to three substituents independently selected from hydroxy, halo (F, Cl, Br, I), C_1-C_4 alkyl, and C_1-C_4-alkoxy;

R_4 is hydrogen or may be joined with R_3 to form a heterocyclic ring selected from piperidine, pyrrolidine or thiazolidine;

R_{14} is hydrogen or methyl;

X is selected from

an O or S atom,

an S atom bearing one or two O atoms,

NR_{13} where R_{13} is selected from hydrogen, C_1-C_4 alkyl, benzyl, phenyl, C_1-C_4 alkanoyl, benzoyl and

$(CH_2)_k$, where k is 0 to 5;

n is 3 or 4;

m is 1; and

pharmaceutically acceptable salts thereof.

4. The compound of claim 2 wherein

R_1 and R_9 are OH;

R_2, R_4, R_5, R_6, R_7, R_8, and R_{14} are hydrogen;

R_3 is selected from

hydrogen, 4-amino-1-butyl, 3-amino-1-propyl, hydroxymethyl, 2-hydroxy-1-propyl, methyl, ethyl, 2-propyl, isobutyl, n-propyl, n-butyl, 2-butyl, methylthioethyl, benzyl, 4-hydroxybenzyl, 4-methoxybenzyl, 3-indolylmethyl, 4-imidazolylmethyl, phenyl, carboxymethyl, carboxyethyl, carboxamidomethyl, and carboxamidoethyl;

X is S; and

n is 3 or 4.

5. The compound of claim 2 wherein

R_1 and R_9 are OH;

R_2, R_4, R_5, R_6, R_7, R_8, and R_{14} are hydrogen;

R_3 is selected from

hydrogen, 4-amino-1-butyl, 3-amino-1-propyl, hydroxymethyl, 2-hydroxy-1-propyl, methyl, ethyl, 2-propyl, isobutyl, n-propyl, n-butyl, 2-butyl, methylthioethyl, benzyl, 4-hydroxybenzyl, 4-methoxybenzyl, 3-indolylmethyl, 4-imidazolylmethyl, phenyl, carboxymethyl, carboxyethyl, carboxamidomethyl, and carboxamidoethyl;

X is SO; and

n is 3 or 4.

6. The compound of claim 2 wherein

R_1 and R_9 are OH;

R_2, R_3, R_4, R_7, R_8, and R_{14} are hydrogen;

R_5 and R_6 are independently selected from hydrogen, methyl, ethyl, propyl, isopropyl, butyl, isobutyl, benzyl, phenyl, 2-trifluoromethylphenyl, 3-trifluoromethylphenyl, 4-trifluoromethylphenyl, 1-naphthyl, 2-naphthyl, 4-biphenyl, and pentafluorophenyl;

X is S; and

n is 3 or 4.

7. The compound of claim 2 wherein

R_1 and R_9 are OH;

R_2, R_3, R_4, R_7, R_8, and R_{14} are hydrogen;

R_5 and R_6 are independently selected from hydrogen methyl, ethyl, propyl, isopropyl, butyl, isobutyl, benzyl, phenyl, 2-trifluoromethylphenyl, 3-trifluoromethylphenyl, 4-trifluoromethylphenyl, 1-naphthyl, 2-naphthyl, 4-biphenyl, and pentafluorophenyl;

X is SO; and

n is 3 or 4.

8. The compound of claim 2 wherein

R_1 and R_9 are OH;

R_2, R_3, R_4, R_5, R_6, and R_{14} are hydrogen;

R_7 and R_8 are independently selected from hydrogen, methyl and phenyl;

X is S; and

n is 3 or 4.

9. The compound of claim 2 wherein

R_1 and R_9 are OH;

R_2, R_3, R_4, R_5, R_6, and R_{14} are hydrogen;

R_7 and R_8 are independently selected from hydrogen, methyl and phenyl;

X is SO; and

n is 3 or 4.

10. The compound of claim 2 wherein

R_1 and R_9 are OH;

R_2, R_4, R_7, R_8, and R_{14} are hydrogen;

R_3 is selected from 4-amino-1-butyl, 3-amino-1-propyl, hydroxymethyl, 2-hydroxy-1-propyl, methyl, ethyl, 2-propyl, isobutyl, n-propyl, n-butyl, 2-butyl, methylthioethyl, benzyl, 4-hydroxybenzyl, 4-methoxybenzyl, 3-indolylmethyl, 4-imidazolylmethyl, phenyl, carboxymethyl, carboxyethyl, carboxamidomethyl, and carboxamidoethyl;

R_5 and R_6 are independently selected from hydrogen, methyl, ethyl, propyl, isopropyl, butyl, isobutyl, benzyl, phenyl, 2-trifluoromethylphenyl, 3-trifluoromethylphenyl, 4-trifluoromethylphenyl, 1-naphthyl, 2-naphthyl, 4-biphenyl, and pentafluorophenyl;

X is S; and

n is 3 or 4.

11. The compound of claim 2 wherein

R_1 and R_9 are OH;

R_2, R_4, R_7, R_8, and R_{14} are hydrogen;

R_3 is selected from 4-amino-1-butyl, 3-amino-1-propyl, hydroxymethyl, 2-hydroxy-1-propyl, methyl, ethyl, 2-propyl, isobutyl, n-propyl, n-butyl, 2-butyl, methylthioethyl, benzyl, 4-hydroxybenzyl, 4-methoxybenzyl, 3-indolylmethyl, 4-imidazolylmethyl, phenyl, carboxymethyl, carboxyethyl, carboxamidomethyl, and carboxamidoethyl;

R_5 and R_6 are independently selected from hydrogen, methyl, ethyl, propyl, isopropyl, butyl, isobutyl, benzyl, phenyl, 2-trifluoromethylphenyl, 3-trifluoromethylphenyl, 4-trifluoromethylphenyl, 1-naphthyl, 2-naphthyl, 4-biphenyl, and pentafluorophenyl;

X is SO; and

n is 3 or 4.

12. The compound of claim 2 wherein

R_1 and R_9 are OH;

R_2, R_4, R_5, R_6, and R_{14} are hydrogen;

R_3 is selected from 4-amino-1-butyl, 3-amino-1-propyl, hydroxymethyl, 2-hydroxy-1-propyl, methyl, ethyl, 2-propyl, isobutyl, n-propyl, n-butyl, 2-butyl, methylthioethyl, benzyl, 4-hydroxybenzyl, 4-methoxybenzyl, 3-indolylmethyl, 4-imidazolylmethyl, phenyl, carboxymethyl, carboxyethyl, carboxamidomethyl, and carboxamidoethyl;

R_7 and R_8 are independently selected from hydrogen, methyl and phenyl;

X is S; and

n is 3 or 4.

13. The compound of claim 2 wherein

R_1 and R_9 are OH;

R_2, R_4, R_5, R_6, and R_{14} are hydrogen;

R_3 is selected from 4-amino-1-butyl, 3-amino-1-propyl, hydroxymethyl, 2-hydroxy-1-propyl, methyl, ethyl, 2-propyl, isobutyl, n-propyl, n-butyl, 2-butyl, methylthioethyl, benzyl, 4-hydroxybenzyl, 4-methoxybenzyl, 3-indolylmethyl, 4-imidazolylmethyl, phenyl, carboxymethyl, carboxyethyl, carboxamidomethyl, and carboxamidoethyl;

R_7 and R_8 are independently selected from hydrogen, methyl and phenyl;

X is SO; and

n is 3 or 4.

14. The compound of claim 2 wherein

R_1 and R_9 are OH;

R_2, R_3, R_4, and R_{14} are hydrogen;

R_5 and R_6 are independently selected from hydrogen, methyl, ethyl, propyl, isopropyl, butyl, isobutyl, benzyl, phenyl, 2-trifluoromethylphenyl, 3-trifluoromethylphenyl, 4-trifluoromethylphenyl, 1-naphthyl, 2-naphthyl, 4-biphenyl, and pentafluorophenyl;

R_7 and R_8 are independently selected from hydrogen, methyl and phenyl;

X is S; and

n is 3 or 4.

15. The compound of claim 2 wherein

R_1 and R_9 are OH;

R_2, R_3, R_4, and R_{14} are hydrogen;

R_5 and R_6 are independently selected from hydrogen, methyl, ethyl, propyl, isopropyl, butyl, isobutyl, benzyl, phenyl, 2-trifluoromethylphenyl, 3-trifluoromethylphenyl, 4-trifluoromethylphenyl, 1-naphthyl, 2-naphthyl, 4-biphenyl, and pentafluorophenyl;

R_7 and R_8 are independently selected from hydrogen, methyl and phenyl;

X is SO; and

n is 3 or 4.

16. The compound of claim 2 wherein

R_1 and R_9 are OH;

R_2, R_4, R_5, R_6, R_7, R_8, and R_{14} are hydrogen;

R_3 is selected from hydrogen, 4-amino-1-butyl, 3-amino-1-propyl, hydroxymethyl, 2-hydroxy-1-propyl, methyl, ethyl, 2-propyl, isobutyl, n-propyl, n-butyl, 2-butyl, methylthioethyl, benzyl, 4-hydroxybenzyl, 4-methoxybenzyl, 3-indolylmethyl, 4-imidazolylmethyl, phenyl, carboxymethyl, carboxyethyl, carboxamidomethyl, and carboxamidoethyl;

X is O; and

n is 3 or 4.

17. The compound of claim 2 wherein

R_1 and R_9 are OH;

R_2, R_4, R_5, R_6, R_7, R_8, and R_{14} are hydrogen;

R_3 is selected from hydrogen, 4-amino-1-butyl, 3-amino-1-propyl, hydroxymethyl, 2-hydroxy-1-propyl, methyl, ethyl, 2-propyl, isobutyl, n-propyl, n-butyl, 2-butyl, methylthioethyl, benzyl, 4-

hydroxybenzyl, 4-methoxybenzyl, 3-indolylmethyl, 4-imidazolylmethyl, phenyl, carboxymethyl, carboxyethyl, carboxamidomethyl, and carboxamidoethyl;

X is NR_{13} where R_{13} is selected from hydrogen, C_1-C_4 alkyl, C_1-C_4 alkanoyl, benzoyl and benzyl; and

n is 3 or 4.

18. The compound of claim 2 wherein

R_1 and R_9 are OH;

R_2, R_4, R_5, R_6, R_7, R_8, and R_{14} are hydrogen;

R_3 is 4-hydroxybenzyl;

X is S; and

n is 3.

19. The compound of claim 2 wherein

R_1 and R_9 are OH;

R_2, R_4, R_5, R_6, R_7, R_8, and R_{14} are hydrogen;

R_3 is 4-hydroxybenzyl;

X is SO; and

n is 3.

20. The compound of claim 2 wherein

R_1 and R_9 are OH;

R_2, R_4, R_5, R_6, R_7, R_8, and R_{14} are hydrogen;

R_3 is isopropyl;

X is S; and

n is 3.

21. The compound of claim 2 wherein

R_1 and R_9 are OH;

R_2, R_4, R_5, R_6, R_7, R_8, and R_{14} are hydrogen;

R_3 is isopropyl;

X is SO; and

n is 3.

22. The compound of claim 2 wherein

R_1 and R_9 are OH;

R_2, R_4, R_5, R_6, R_7, R_8, and R_{14} are hydrogen;

R_3 is 2-butyl;

X is S; and

n is 3.

23. The compound of claim 2 wherein

R_1 and R_9 are OH;

R_2, R_4, R_5, R_6, R_7, R_8, and R_{14} are hydrogen;

R_3 is 2-butyl;

X is SO; and

n is 3.

24. The compound of claim 2 wherein

R_1 and R_9 are OH;

R_2, R_5, R_6, R_7, R_8, and R_{14} are hydrogen;

R_3 and R_4 are joined together to form a pyrrolidine ring;

X is S; and

n is 3.

25. The compound of claim 2 wherein

R_1 and R_9 are OH;

R_2, R_5, R_6, R_7, R_8, and R_{14} are hydrogen;

R_3 and R_4 are joined together to form a pyrrolidine ring;

X is SO; and

n is 3.

26. The compound of claim 2 wherein

R_1 and R_9 are OH;

R_2, R_4, R_5, R_6, and R_{14} are hydrogen;

R_3 is 4-hydroxybenzyl;

R_7 and R_8 are methyl;

X is S; and

n is 3.

27. The compound of claim 2 wherein

R_1 and R_9 are OH;

R_2, R_4, R_5, R_6, and R_{14} are hydrogen;

R_3 is 4-hydroxybenzyl;

R_7 and R_8 are methyl;

X is SO; and

n is 3.

28. The compound of claim 2 wherein

R_1 and R_9 are OH;

R_2, R_4, R_5, R_6, and R_{14} are hydrogen;

R_3 is isopropyl;

R_7 and R_8 are methyl;

X is S; and

n is 3.

29. The compound of claim 2 wherein

R_1 and R_9 are OH;

R_2, R_4, R_5, R_6, and R_{14} are hydrogen;

R_3 is isopropyl;

R_7 and R_8 are methyl;

X is SO; and

n is 3.

30. The compound of claim 2 wherein

R_1 and R_9 are OH;

R_2, R_3, R_4, R_5, R_6, and R_{14} are hydrogen;

R_7 and R_8 are methyl;

X is S; and

n is 3.

31. The compound of claim 2 wherein

R_1 and R_9 are OH;

R_2, R_3, R_4, R_5, R_6, and R_{14} are hydrogen;

R_7 and R_8 are methyl;

X is SO; and

n is 3.

32. The compound of claim 2 wherein

R_1 is NH_2;

R_9 is OH;

R_2, R_4, R_5, R_6, R_7, R_8, and R_{14} are hydrogen;

R_3 is selected from hydrogen, 4-amino-1-butyl, 3-amino-1-propyl, hydroxymethyl, 2-hydroxy-1-propyl, methyl, ethyl, 2-propyl, isobutyl, n-propyl, n-butyl, 2-butyl, methylthioethyl, benzyl, 4-hydroxybenzyl, 4-methoxybenzyl, 3-indolylmethyl, 4-imidazolylmethyl, phenyl, carboxymethyl, carboxyethyl, carboxamidomethyl, and carboxamidoethyl;

X is S; and

n is 3 or 4.

33. The compound of claim 2 wherein

R_1 is NH_2;

R_9 is OH;

R_2, R_4, R_5, R_6, R_7, R_8, and R_{14} are hydrogen;

R_3 is selected from hydrogen, 4-amino-1-butyl, 3-amino-1-propyl, hydroxymethyl, 2-hydroxy-1-propyl, methyl, ethyl, 2-propyl, isobutyl, n-propyl, n-butyl, 2-butyl, methylthioethyl, benzyl, 4-hydroxybenzyl, 4-methoxybenzyl, 3-indolylmethyl, 4-imidazolylmethyl, phenyl, carboxymethyl, carboxyethyl, carboxamidomethyl, and carboxamidoethyl;

X is SO; and

n is 3 or 4.

34. The compound of claim 2 wherein

R_1 and R_9 are OH;

R_2, R_4, R_5, R_6, R_7, R_8, and R_{14} are hydrogen;

R_3 is selected from hydrogen, 4-amino-1-butyl, 3-amino-1-propyl, hydroxymethyl, 2-hydroxy-1-propyl, methyl, ethyl, 2-propyl, isobutyl, n-propyl, n-butyl, 2-butyl, methylthioethyl, benzyl, 4-hydroxybenzyl, 4-methoxybenzyl, 3-indolylmethyl, 4-imidazolylmethyl, phenyl, carboxymethyl, carboxyethyl, carboxamidomethyl, and carboxamidoethyl;

X is $(CH_2)_k$ where k is 0,1 or 2; and

n is 3 or 4.

35. The compound of claim 2 wherein

R_1 and R_9 are OH;

R_2, R_4, R_5, R_6, R_7, R_8, and R_{14} are hydrogen;

R_3 is 4-hydroxybenzyl;

X is CH_2; and

n is 3.

36. The compound of claim 2 wherein

R_1 and R_9 are OH;

R_2, R_4, R_5, R_6, R_7, R_8, and R_{14} are hydrogen;

R_3 is isopropyl;

X is CH_2; and

n is 3.

37. The compound of claim 2 wherein

R_1 and R_9 are OH;

R_2, R_4, R_5, R_6, R_7, R_8, and R_{14} are hydrogen;

R_3 is 2-butyl;

X is CH_2; and

n is 3.

38. The compound of claim 2 wherein

R_1 and R_9 are OH;

R_2, R_5, R_6, R_7, R_8, and R_{14} are hydrogen;

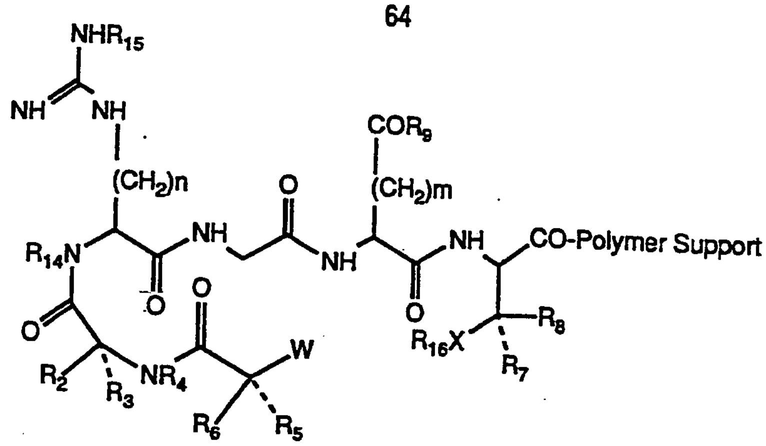
R_3 and R_4 are joined together to form a pyrrolidine ring;

X is CH_2; and

n is 3.

39. A process for preparing the compound of claim 2 which comprises:

(a) cyclizing an intermediate of Formula IV



IV

wherein

R_1-R_9, n and m are as defined in claim 2,

W is Br, Cl, or I,

R_{14} is selected from hydrogen, C_1-C_6 alkyl, and C_6-C_{12} aryl

R_{15} is 2,2,5,7,8-pentamethylchroman-6-sulfonyl,

R_{16} is hydrogen, and

X is S;

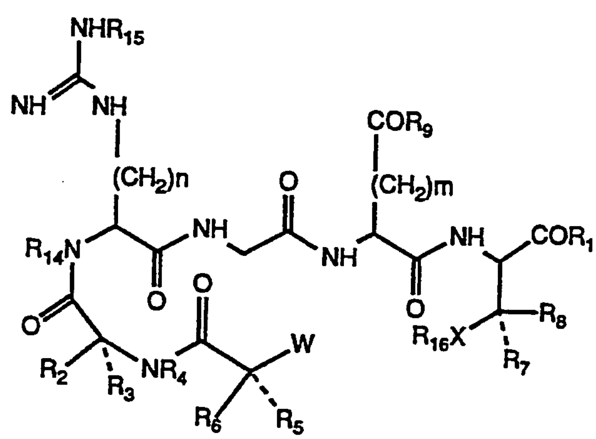
(b) cleaving the desired product from the resin;

(c) cleaving any protecting groups; and

(d) purifying and isolating the more biologically active isomer.

40. A process for preparing the compound of claim 2 which comprises:

(a) cyclizing an intermediate of Formula VI



VI

wherein

R_1-R_9, n and m are as defined in claim 2,

W is Br, Cl, or I,

R_{14} is selected from hydrogen, C_1-C_6 alkyl, and C_6-C_{12} aryl

R_{15} is 2,2,5,7,8-pentamethylchroman-6-sulfonyl,

R_{16} is H, and

X is S;

(b) cleaving any protecting groups; and

(c) purifying and isolating the more biologically active isomer.

41. A pharmaceutical composition comprising a pharmaceutically acceptable excipient and the compound of Claim 1.

42. A method for inhibiting platelet aggregation which method comprises administering a platelet aggregation inhibiting amount of the compound of Claim 1.

43. A method for reducing platelet aggregation in a mammal, comprising administering a pharmaceutically effective amount of the composition of matter as defined by claim 1 to said mammal.

44. The method of claim 43, further comprising administering said composition of matter to said mammal in admixture with a pharmaceutically acceptable carrier.

45. A method for treating a mammal who has an increased propensity for thrombus formation, comprising administering a pharmaceutically effective amount of the composition of matter as defined by claim 1 to said mammal.

46. A composition of matter for reducing platelet aggregation in a mammal, comprising the composition of matter as defined by claim 1.

47. A composition of matter for treating a mammal who has an increased propensity for thrombus formation, comprising the composition of matter as defined by claim 1.

48. A composition of matter for inhibiting fibrinogen binding to platelets in a mammal, comprising the composition of matter as defined by claim 1.

49. A composition of matter comprising the compositions of claim 1 wherein R_1 is C_1-C_6 alkoxy which includes branched and unsaturated alkyl groups.

50. A composition of matter comprising the compositions of claim 1 wherein R_9 is C_1-C_6 alkoxy which includes branched and unsaturated alkyl groups.

51. A composition of matter comprising the compositions of claim 1 wherein R_1 and R_9 are both C_1-C_6 alkoxy which includes branched and unsaturated alkyl groups.

52. A method of treating a mammal who has an increased propensity for thrombus formation which comprises administering to said mammal a therapeutically effective amount of the composition of claim 51, 52, or 53 wherein the R_1 and R_9 alkoxy groups are hydrolyzed following administration to said mammal.

53. A method for treating a mammal who has an increased propensity for thrombus formation, comprising administering a pharmaceutically effective amount of the composition of matter as defined by claim 1 in combination with a thrombolytic agent.

54. A method for treating a mammal who has an increased propensity for thrombus formation, comprising administering a pharmaceutically effective amount of the composition of matter as defined by claim 1 in combination with an anticoagulant.

55. A method for treating a mammal who has an increased propensity for thrombus formation, comprising administering a pharmaceutically effective amount of the composition of matter as defined by claim 1 following angioplasty.

56. A cyclic peptide containing the tripeptide sequence Arg-Gly-Asp and a thioether linkage in the cycle.

57. The cyclic peptide of claim 57 wherein the cyclic peptide contains 5 amino acids in the cycle.

58. The cyclic peptide of claim 57 wherein the cycle contains 17 or 18 atoms in a ring.

59. The cyclic peptide of claim 58 wherein the cyclic peptide contains at least one D-α-amino acid.

60. The cyclic peptide of claim 59 wherein the D-amino acid is in any position in the cycle except the Arg-Gly-Asp sequence.

61. The cyclic peptide of claim 57 wherein the sulfur of the thioether linkage is bonded to at least one oxygen.

62. A cyclic pentapeptide containing the tripeptide sequence Arg-Gly-Asp wherein the cycle comprises a peptide linkage through an amino acid sidechain.

63. The cyclic peptide of claim 62 wherein the peptide linkage is through the 6 carboxyl of 2-amino-1,6-hexanedioic acid.

64. The cyclic pentapeptide of claim 63 wherein the cycle contains 17 or 18 atoms in a ring.

INTERNATIONAL SEARCH REPORT

International Application No PCT/US 90/03788

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [6]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC^5: C 07 K 7/54, C 07 K 7/56

II. FIELDS SEARCHED

Minimum Documentation Searched [7]

Classification System	Classification Symbols
IPC^5	C 07 K

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

Category [*]	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No. [13]
Y	Peptides, Chemistry, Structure and Biology, Proceedings of the eleventh American Peptide Symposium, 9-14 July 1989, La Jolla, California, US, Escom, (Leiden, NL), F. El-Fahail Ali et al.: "Structure-activity studies toward the improvement of antiaggregatory activity of Arg-Gly-Asp-Ser (RGDS)", pages 94-96 see pages 94-95, results and discussion (cited in the application) --	1-4,6,8,30, 32,34,46-49, 56-58,62
Y,P	WO, A, 89/07609 (UPJOHN) 24 August 1989 see page 1, line 25 - page 2, line 34; page 8, line 22 - page 9, line 37 --	1-4,6,8,30, 32,34,46-49, 56-58,62
Y,P	WO, A, 90/02751 (ASAHI GLASS COMPANY) 22 March 1990 ./.	1-4,6,8,30, 32,34,46-49,

* Special categories of cited documents: [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
10 October 1990	-6 NOV 1990
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT OFFICE	MISS D. S. KOWALCZYK

Form PCT/ISA/210 (second sheet) (January 1985)

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category *	Citation of Document, [11] with indication, where appropriate, of the relevant passages	Relevant to Claim No.
	see page 1, abstract; page 42, claims 1-2	56-58,62
	--	
Y	The Journal of Biological Chemistry, volume 262, no. 36, 25 December 1987, (Baltimore, US), M.D. Pierschbacher et al.: "Influence of Stereochemistry of the sequence Arg-Gly-Asp-Xaa on binding specificity in cell adhesion", pages 17294-17298 see page 17295, lines 3-15; page 17296, lines 12-28; page 17297, line 6 - page 17298, line 3	

International Application No. PCT/US 90/03788

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

V. [X] OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE [1]

This international search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1. [X] Claim numbers ...xx... because they relate to subject matter not required to be searched by this Authority, namely:

xx Claims 42-45, 52-55
See PCT Rule 39.1(iv);
Methods for treatment of the human or animal body, by means of surgery of therapy, as well as diagnostic methods.

2. [ ] Claim numbers ...., because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. [ ] Claim numbers.........., because they are dependent claims and are not drafted in accordance with the second and third sentences of PCT Rule 6.4(a).

VI. [ ] OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING [2]

This International Searching Authority found multiple inventions in this international application as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.
2. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:
3. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:
4. [ ] As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest

[ ] The additional search fees were accompanied by applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO. US 9003788
SA 38932

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report.
The members are as contained in the European Patent Office EDP file on 26/10/90
The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO-A- 8907609	24-08-89	AU-A- 3040589	06-09-89
WO-A- 9002751	22-03-90	None	

EPO FORM P0479

For more details about this annex : see Official Journal of the European Patent Office, No. 12/82